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CORRELATION OF OILS AND OIL PRODUCTS BY GAS CHROMATOGRAPHY

by

O. Zafiriou, M. Blumer and J. Myers

July 1972

TECHNICAL REPORT

Prepared for the Office of Research and Monitoring, Environmental Protection Agency, under Contract 15080 HEC.

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The method is suitable for routine use for weeks-old oil samples, and for monitoring levels of hydrocarbons in organisms and sediments. Slightly modified, it can be used in low-level and research work studying the fate and effects of petroleum hydrocarbons.

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J. M. Hunt, Chairman
Department of Chemistry

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ABSTRACT

Environmental samples of oil and oil products can be correlated with possible sources by comparison of their stable compositional features. Procedures for correlating samples with suspected sources by gas chromatographic analysis under standardized conditions are presented in manual form. A high rate of success for the method in realistic situations is estimated by analyses of artificially aged oils and of potential spill sources found in Greater New York Harbor and Portland, Maine.

The method is suitable for routine use for weeks-old oil samples, and for monitoring levels of hydrocarbons in organisms and sediments. Slightly modified, it can be used in low-level and research work studying the fate and effects of petroleum hydrocarbons.

INTRODUCTION

This Technical Report describes a method of correlating oil spills with suspected sources by gas chromatographic analysis. The method was developed at Woods Hole Oceanographic Institution by the authors under Environmental Protection Agency Contract 15080 HEC.

The Introduction establishes the basis of all oil correlation methods and estimates the suitability of the gas chromatographic method for spill-source correlations in various situations. Though the Introduction is non-technical, it *should be read by anyone intending to use the method or its results.*

CHEMISTRY OF PETROLEUM

Petroleum and most high-volume petroleum products are extremely complex mixtures of chemical compounds. Many classes of compounds are present in petroleum; each class is represented by many components. For example, *hydrocarbons* are a major class of constituents of petroleum. The hydrocarbons include the subclasses *normal, branched, cyclic, saturated,* and *aromatic* hydrocarbons. The subclass of normal hydrocarbons - one of the smallest ones - is represented by 10 - 60 individual members in many oils and oil products. Most other subclasses have many more representatives. An entirely similar degree of complexity applies to other, completely different classes of compounds, such as *heavy metals, heteroaromatics, carboxylic acids, mercaptans.*

Thus, *the fundamental aspect of oil chemistry is complexity of composition* both among and within categories. This complexity is the basis of *any* method for chemically identifying oils, and is based on the following facts:

1. Oils are formed by very complex biological, geological and chemical processes involving the growth, death, burial and transformation of living matter, and the collecting of economically recoverable quantities of these transformation products in suitable geological structures.
2. The complexity of oils is so great and the factors influencing their composition are so numerous that two oil samples with identical composition can only arise if all contributing factors have been identical: oils with identical compositions have identical histories.

FINGERPRINTING AND CORRELATION

Point 2 is the basis of oil "correlation", "fingerprinting" or "passive tagging". In principle, *two samples of oil with truly identical chemical composition must have identical histories.* This relationship is the "fingerprint principle".

The complexity of oil structure, which yields the "fingerprint principle" also gives rise to an important limitation: oil and oil products are so complex that no typical oil has ever been completely analyzed. All feasible analyses of oils are partial, and few actually account for more than a few percent of the total weight of material analyzed. Since analyses are partial and subject to errors of many types, the full promise of the "fingerprint principle" cannot be realized. Experimental error introduces inaccuracies in the levels of components analyzed; also, the incompleteness of the analysis may mean that the components in which two oils differ most are not determined. In practice, then, very similar oils may not be distinguishable. All oil correlation is fundamentally the *comparison of two*

or more oil samples. The strongest statement that can be made is that the oils compared are definitely different or that they are not distinguishable by the method used.

A second consequence of the "fingerprint principle" is: only oils with *identical* histories have truly identical compositions. In most environmental applications, fresh oils suspected as spill sources must be compared with environmentally altered samples. These two samples have different histories. They are therefore expected to have different compositions. The differences in oil composition caused by environmental exposure (effects of wind, water, organisms, sediments, etc.) are known as *weathering*. In order to trace the source of the environmental sample, one must be able to specify the differences between source and spill which can reasonably be expected to arise from weathering. Any significant differences not attributable to weathering establish that the samples are different (that is, their histories had been different even before one was spilled.)

In summary, "fingerprint" analyses can theoretically correlate all identical oils. Errors and incompleteness of analysis limit this capability, and weathering introduces further differences between an environmentally exposed oil and its source. The net effect of these factors is that chemical comparison of oil samples leads to a unique source-sample correlation only if *the spill and all possible sources in the area are analyzed, if all but one possible source shows compositional differences from the spill sample which cannot arise by weathering alone, and if the remaining possible source differs from the spill sample only in ways attributable to weathering.*

WATER QUALITY MANAGEMENT

Clearly the practical effectiveness of an oil correlation method cannot

TABLE I

CORRELATION OF SOURCE OILS AND SIMULATED SPILLS BY GAS CHROMATOGRAPHY

PREPARED BY EPA		CORRELATED BY WOODS HOLE *	RESULT
17 SOURCE OILS:	35 SIMULATED SPILLS:	26 UNIQUE CORRELATIONS	ALL CORRECT
8 CRUDES	EXPOSURES:	6 "PROBABLE" CORRELATIONS (A SECOND SOURCE POSSIBLE)	1 ERROR**
5 FUELS	1, 3, 30 DAYS		
4 RE-REFINED	FRESHWATER, SALTWATER, BEACH SAND	3 NO DEFINITE OR PROBABLE CORRELATIONS FOUND	NO CORRELATION EXISTS ***
	WITH OR WITHOUT 1 of 4 SPILL CONTROL CHEMICALS ADDED.		

* See Appendix 5

** Error attributable to presence of oil-based spill control chemical

*** The source of these samples was not supplied to Woods Hole by E.P.A.

be determined from first principles alone. The method developed and presented in this Manual was designed to take maximum advantage of the "fingerprint" variability and minimize the effects of analytical inaccuracy, of the incompleteness of analysis, and of weathering effects. The success of these efforts is measured by how well the method works in several settings realistically related to water quality management.

In a first test of the gas chromatographic method, EPA personnel at Edison, New Jersey exposed a variety of commonly encountered oils and oil products to environmental weathering under different conditions for varying lengths of time. Several spill-control chemicals (6% by weight) were also added to some samples. Sixteen of the seventeen source oils used were sent to Woods Hole along with the 35 simulated spill samples. Samples were number coded and identified only as potential sources or simulated spills. The results of correlating these samples (Appendix 5) by gas chromatography are presented in Table 1. For each spill sample, all sixteen source samples were considered. Over 10^{42} possible sets of correlations exist, so that correct identification by chance is nearly impossible. As Table 1 shows, a definite and correct correlation was made in 74% of the cases; furthermore, only one "probable" correlation was incorrect. Significantly, EPA had prepared three variants of a sample without supplying the corresponding source oil; the analysis indicated that none of the given sources was likely for these samples.

Since this comparison of sources and simulated spills involved a variety of oils and weathering conditions within the range encountered in actual water quality management, the method has an excellent chance of correlating real spills with their sources. In particular, *no incorrect "definite"*

TABLE 2

PER CENT SUCCESS OF GAS CHROMATOGRAPHIC CORRELATION OF "SPILLS" AND "SOURCES"

	% SUCCESS = % OF SPILL SAMPLES CORRELATED WITH ONE AND ONLY ONE SOURCE SAMPLE[a]
% SUCCESS BY CATEGORIES:	
CRUDES	86
PRODUCTS	73
1 - 3 DAY EXPOSURE	80
30 DAY EXPOSURE	72
NO ADDITIVES	75
WITH ADDITIVES	73
OVERALL:	81

[a]See Table 1. ("Spills" supplied without corresponding source oils not considered.) Success = 26/32.
All correlations made were correct.

correlations were made. Table 2 breaks down the performance of the correlation method by the variables involved. Within the range of situations covered, the success rate (spill sample corresponds to 1 and only 1 weathered possible source) does not vary significantly and is about 70-80%.

The data of Tables 1 and 2 strongly suggest that the method may be a useful tool in water quality management. However, some alternative combinations of the seventeen source oils of Table 1 would have lowered considerably the success rate; some would have enhanced it. The virtually perfect performance of the method in this trial experiment cannot be expected to hold for all cases;had EPA chosen some other sources to generate several weathered spill samples, the success rate could have been much lower. On the other hand, comparison of each spill sample with sixteen possible sources (when there were actually 17 possible sources) is a much more complex situation than many real environmental situations present. The fewer the possible sources, the simpler correlation is; and generally, the higher the success rate.

A second test of the potential method has been made by obtaining representative samples of the oils occurring in various areas and analyzing them. Table 2 clearly shows that mild weathering - exposure for up to 30 days - has minimal effects on the ability of the method to correlate oils. Therefore, success in uniquely correlating oil spills after brief weathering depends mainly on distinguishing all possible sources from one another.

In light of the small effect of short-term weathering on correlation success rate shown in Table 2, the performance of an oil analysis method

TABLE 3

ABILITY TO DISTINGUISH AMONG CRUDE OILS FROM PORTLAND, MAINE

# OF CRUDE OILS ANALYZED:	22
% DISTINGUISHED FROM:	
ALL OTHER OILS	27
ALL BUT 1 OTHER OIL	50
ALL BUT 2 OTHER OILS	13
ALL BUT 3 OTHER OILS	5
ALL BUT 4 OTHER OILS	5

in a given locale can be estimated by determining how many of the possible sources in the area can be distinguished from one another. That is, *practical performance will tend to be limited by the presence of similar oils, and not by short-term weathering.*

The port of Portland, Maine transships the cargoes of over 450 oil tankers per year. The Maine Environmental Improvement Commission has provided us with samples of the cargoes of 22 tankers, representing all arrivals during a 2 week time period. Crude oil traffic far exceeds other types in Portland, and we assume the arrivals during this period are typical. To reduce the number of required analyses to manageable proportions, we estimated statistically whether some oils could be distinguished from others; these estimates cover only a few cases and the results presented in Table 3 are close to those that would be achieved by further analyses of all possible pairs.

The crude oils arriving in Portland cannot be uniquely distinguished in a majority of the cases; in practice, tankers arriving from the same oil shipment port bear virtually indistinguishable (by this method) cargoes. Nevertheless, even if all arrivals over two full weeks must be considered, one-quarter of the cargoes were unique and one-half could only arise from one of two ships. For samples that are not unique, useful narrowing of the field of possibilities is straightforward.

The Greater New York Harbor area is a complex refinery center and primarily a "sink" in which a great variety of oil *products* are consumed. The U. S. Coast Guard obtained for us samples of the cargoes of some 42 ships, barges, and dock facilities in this area; the purpose of their in-

TABLE 4

ABILITY TO DISTINGUISH AMONG 30 OILS AND OIL PRODUCTS[a] FROM GREATER NEW YORK HARBOR

PRODUCT TYPE	NO. OF SAMPLES	ASSIGNMENT
GASOLINE	12	NOT ANALYZED
#2 FUEL OIL	14	7 UNIQUE
		3 PAIRS OF INDISTINGUISHABLE OILS
		1 TRIO OF INDISTINGUISHABLE OILS
#4 FUEL OIL	4	ALL UNIQUE
#6 FUEL OIL	8	ALL UNIQUE
MISCELLANEOUS:		
KEROSENE	1	UNIQUE
MARINE DIESEL	1	UNIQUE
NIGERIAN CRUDE	1	UNIQUE
ASPHALT	1	UNIQUE
OVERALL:		77% UNIQUE
		20% INDISTINGUISHABLE PAIRS
		3% INDISTINGUISHABLE TRIPLETS

[a]Each oil was analyzed and compared with the 29 others to determine distinguishability.

spection is to assure compliance with hazardous cargo handling regulations and their collection is relatively nonselective and covers most major refinery docks and other shipment points in the area. The same type of model correlation by distinguishing these oils from one another (see Portland, Maine analysis above) was applied to the 30 non-gasoline samples from Greater New York Harbor. Table 4 shows the results.

The model correlations in Greater New York Harbor are excellent. All samples can be classified according to type by the analysis, and except for some #2 fuel oils, all samples are uniquely identifiable. The correlation for #2 fuel oil is also high - 50% correlate uniquely, and most of the pairs were collected on widely separated locations or at different times.

PRACTICABILITY

Many methods have been proposed for correlating environmental oil pollutants with their sources. For example, a complex, expensive, slow activation analysis scheme is claimed (Lukens, 1971) to have extremely high discriminating power; also, extensive geochemical analysis of samples has been suggested (Koons, 1972). Both of these methods are undoubtedly of some value. Both invest a large amount of time and effort in each sample, or use highly specialized and expensive equipment.

In contrast, the gas chromatographic method can be set up for less than $5,000 in equipment and supplies, and run by a competent BS chemical technician with some experience in gas chromatography. Analyses of bulk oil samples require about 15 minutes of technician time and 1 hour of instrument time. This flexibility makes GC analysis useful not only for spill-source correlations, but also for monitoring environmental oil levels

routinely, and for research involving the environmental fate of oil. Of course, such work requires more time, skill and effort than simple correlations, especially for sample preparation. Procedures are included in this Manual for treating sediments, dilute oil/water samples, and oil in organisms.

As an example of the research potential of gas chromatographic analysis the fate of an oil spill in West Falmouth, Massachusetts has been followed successfully for over two years. The location, concentrations and compositional alteration of #2 fuel oil in marine and marsh sediments and in organisms were determined and correlated with biological data (Blumer, 1970, 1971, 1972; Sanders, 1972). The gas chromatographic techniques presented in this Manual are as reproducible and of higher resolution than those required for the West Falmouth study.

A second advantage of gas chromatographic analysis is that the resultant chromatogram can guide the selection of applicable techniques for more detailed analysis. For example, similar oils might be recognized as fuel oils, which are blended to meet S content requirements; distinguishing these oils by sulfur analysis would not be promising as they would tend to cluster very near the legal maximum S content. A section explaining the use of gas chromatographic information to indicate suitable further analysis is included in this manual.

A third advantage of gas chromatographic analysis is that the sample is essentially separated according to the evaporating tendency of its constituents. Since evaporation is the most active environmental force altering oil composition (especially on a short term basis), this separation

clearly indicates evaporation effects and indicates the unaltered portion of an oil "fingerprint".

Several disadvantages traditionally associated with the gas chromatographic analysis of oil pollutants have been overcome in this method. For example, the Western Oil and Gas Association concluded (Kreider, 1971) that it was not possible to compare oil samples except on the same instrument, using the same column, on the same day. We have been able to remove these limitations for all but the closest comparisons. The same group has objected that relatively high resolution columns, as used in this study, have very limited lifetime when subjected to repeated oil sample analysis; however, the columns used in our study have shown a useful life of at least 200 analyses.

This Manual presents a brief introduction to the chemistry of oil and oil products, the geochemistry of the processes forming and altering oil - including weathering - and the approaches necessary to optimize oil analysis by gas chromatography. The method of analysis developed is presented in detail.

CORRELATION OF OILS AND OIL PRODUCTS BY GAS CHROMATOGRAPHY

Analysis of standard samples to demonstrate capability is an essential part of the following method. All investigators planning to utilize the method for oil-rich samples should obtain and analyze the standard oil sample available upon request from the senior author or from the Edison, New Jersey E.P.A. laboratory.

Investigators planning to analyze materials extracted from organisms should obtain and analyze the standard organism sample (a cod-liver oil spiked with the standard oil, provided as a benzene-methanol solution). Investigators planning to analyze sediments should obtain and analyze a standard sediment sample consisting of a subsample of a homogenized, frozen, oil-contaminated marsh or bottom sediment.

The organism and sediment samples are obtainable upon request from the authors, accompanied by payment of shipping costs and a gas chromatogram or facsimile thereof showing the analysis of the standard oil by this method, or under specified conditions.

Sufficient standard oil is available to supply approximately 1,000 laboratories; spiked cod liver oil, 100 laboratories; sediment polluted with #2 fuel oil, 100 laboratories.

Laboratories not following these recommendations have not performed a minimal capability demonstration, and are not utilizing the method presented herein.

PETROLEUM: CHEMISTRY

Many excellent articles and books discuss the origin and chemistry of petroleum; individuals with major responsibility in the area of oil pollution should be familiar with this literature. Several useful articles are referenced in Appendix I . This section presents a brief summary of the main features of oil chemistry and geochemistry. For the purpose of this Manual, "oil" or "petroleum" is a carbon-rich liquid which can be made to flow from the earth; this definition excludes natural gas and pit-mined asphalt.

The major constituents of oil span the entire molecular weight (MW) range from small molecules (He, CH_4,) to very high molecular weight polymers and polymer aggregates. Nevertheless, the low molecular weight compounds (MW below ca. 2,000; "maltenes")are usually distinguished from the higher molecular weight polymeric material ("asphaltenes").

The predominant element in both these classes is carbon. The high molecular weight polymer is rich in linked aromatic structures, including heteroaromatic compounds of N, S and O, and metal-organic complexes; little is known of the structure composition of the polymeric fraction. The forces between individual polymer molecules are strong, so that much of the high MW fraction is essentially a single, giant molecule. Analysis of this fraction involves its destruction in varying degrees.

In contrast, the lower molecular weight fraction is an extremely complex mixture of mutually dissolved carbon compounds which offer much greater possibilities for chemical analysis than the high molecular weight material. The relative proportions of the two fractions vary greatly

among oils and also among oil products. The low molecular weight fraction responsible for the fluidity of oils is always present (except in some tars and asphalt) in sufficient amount for GC analysis.

In addition to the major high and low MW components mentioned above, oils contain many other lesser constituent classes, such as organic and mineral particles and emulsified water and its solutes, in minor and highly variable amounts.

The lower molecular weight fraction of oil is a complex mixture of compounds which offers the greatest promise for correlating oil; it is also chemically and geochemically the best understood fraction. Table 5 lists some of the major types of these compounds found in many oils. Each type is represented by many individual compounds in most oils. Most of these compounds are mutually soluble, stable, and relatively water-insoluble substances. Their evaporating tendencies vary greatly with molecular size; some components are permanent gases at normal temperatures (methane, ethane); others are high-melting solids (longer-chain normal paraffins).

This Manual presents a method of analyzing the oils by gas chromatography on a non-polar column and detection by flame ionization. The advantages and disadvantages of this approach are discussed later; it partially separates the low molecular weight oil fractions according to their individual evaporating tendencies. Individual substances elute from the column and are detected in an order closely related to their boiling points. The gas chromatogram thus yields information about a major fraction of an oil by separating it according to a property--volatility--which is predominant in environmental weathering (evaporation) and in

TABLE 5

PETROLEUM CONSTITUENTS

LOW MOLECULAR WEIGHT CLASS

Hydrocarbons

Saturated	Aromatic
Straight chain	1 - 6+ rings
Isoprenoid	Alkylated
Branched chain	Polyalkylated
Cyclic, polycyclic	Cycloalkylated
	Metal-organic complexes

"NSO" Compounds

Mercaptans
Thiophenes
Aryl thiophenes
Indoles, N heterocycles
Carboxylic Acids
Etc.

HIGH MOLECULAR WEIGHT "ASPHALTENES"

Structural Units

Chains
Crosslinks
Multi-ring aromatics
Heterocycles
Metals

refining (distillation) of oils. As one might expect, these gas chromatograms of oils are useful in gaining an understanding of oil and oil compositions. Appendix 2 contains illustrations of gas chromatograms of a variety of crude oils and oil products. All gas chromatograms have been determined under comparable conditions; in all cases the vertical axis is the detector response, closely proportional to the amount of carbon-bearing material eluted, and the horizontal axis represents the time elapsed during the temperature-programmed run. Figure 1 indicates the positions of some identified peaks on a chromatogram of a standard #2 fuel oil; the tick marks on chromatograms indicate the expected peak position for normal heptadecane ($n\text{-}C_{17}$). The "baseline" signal is determined separately and is subtracted from the sample signal to determine the contribution of the oil to the signal.

Even casual inspection of the gas chromatograms of crude oils in Appendix 2 reveals certain basic features, with great variety in the relative contribution of these features. The oils all show a signal greater than the baseline over wide ranges of elution temperature. This signal is caused by the continuous elution of an extremely complex overlapping of peaks; each substance is in very small amounts. The sum of the peaks as sensed by the detector is a relatively smooth curve with minor variations in intensity. A particularly good example of this feature is given by Bachaquero crude oil (Figure 2) in which this characteristic accounts for almost the entire signal.

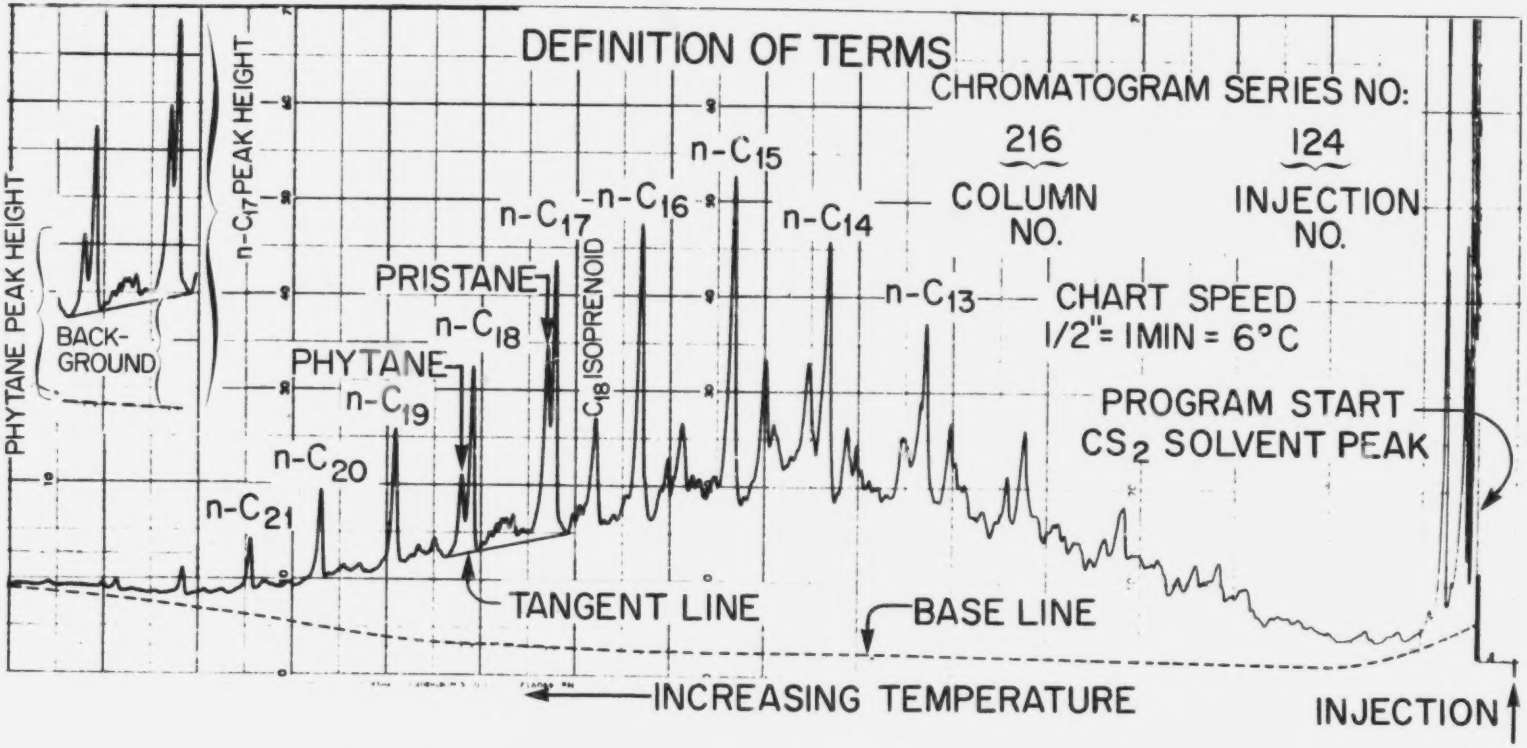

Figure 1. Chromatogram Showing Definition of Terms

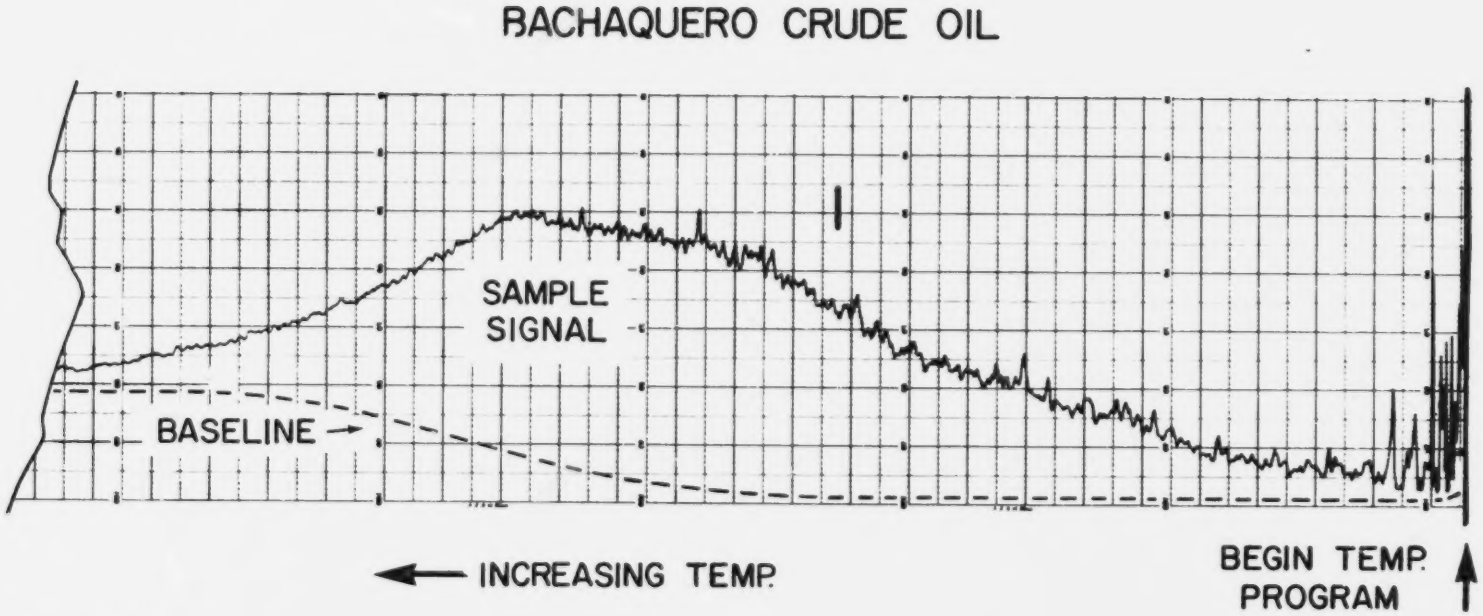

Figure 2. Chromatogram of Bachaquero Crude Oil

The complexity of this mixture of compounds should not be underestimated; it is typical of crude oils generally. For the portion of oil which does not rapidly evaporate or dissolve, no presently feasible columns can perform a complete separation, with the detector signal returning to the baseline between separated peaks. For example, the very best capillary columns are capable of resolving up to about 100 compounds in the interval between two successively eluting normal hydrocarbons. However, it is known from oil geochemistry that thousands of substances occur in the C_{15+} region.

The Introduction emphasized that all oil analyses are partial analyses; in the case of gas chromatography, this incompleteness can be seen in the chromatogram itself. The "unresolved envelope" is *not* an experimental artifact or an indication of poor technique; it is a consequence of oil composition.

The gas chromatograms of many crude oils show a series of well-resolved peaks in addition to the complex mixture of substances just discussed. Chemical fractionation of oils shows that the generally prominent, series of peaks (Chromatograms 7, 16, 20, 30, etc.) is due to the presence of an extended homologous series of normal straight chain hydrocarbons. *No other prominent homologues yielding such an extended series of discrete peaks has ever been found in any crude oil.* The size of these peaks relative to each other and to the "unresolved envelope" varies from oil to oil, and also according to the column resolution.

In addition to these discrete normal hydrocarbon peaks, several

other relatively large peaks occur in many crude oils, particularly those due to the isoprenoid hydrocarbons *pristane* and *phytane* (Figure 1). The origin of these substances is discussed later; they occur at different levels in different oils and are relatively little affected by weathering.

The features discussed above--broad envelope, normal hydrocarbon peaks, pristane and phytane peaks--account for the appearance of most of the crude oil chromatograms in Appendix 2. They are the major features compared in the correlation of oil samples. Many other peaks of intermediate size occur in many oil chromatograms and can be used for oil correlation as well; there is no absolute distinction between the partly resolved, smaller peaks and the larger ones. However, in practice, oils with different patterns of smaller peaks generally have different patterns for the larger ones. The smaller features therefore serve more as a confirmatory check of a postulated correlation than as a starting point.

It is necessary to clarify some terms to avoid confusion. The peaks in an oil chromatogram will frequently be called "pristane peak" or simply "pristane", or "n-C_{18}" (meaning normal octadecane). This nomenclature is a highly useful shorthand. The peaks named in this manner occur at the same location in an oil chromatogram as do the separately analyzed authentic substances after which they are named. The authentic substances have been isolated from many oils. However, the presence of peaks at these expected locations does not <u>prove</u> the structure of the substance found. The peaks in an oil chromatogram do not

represent pure substances. The signal is a sum including a substantial contribution from the "unresolved envelope"; in the case of pristane and phytane, several closely related isomers may also be eluting together. It is not possible to make an exact separation of the signal into "peak" and "background" components without considerable chemical fractionation. We emphasize that the facts just outlined do not diminish the value of correlations, which simply involve the presence or absence of differences. Knowing the chemical identity of major contributing components of peaks is important in assessing the effects of weathering.

The main features and comments which have been made about crude oils apply, with slight modification, to many oil products as well. Many fuel and bunker oils (Appendix 2) are made by the distillation of crude oils, along with additions of other materials in small quantities. These products do not necessarily resemble any particular crude oil, as many crudes may become admixed in the manufacturing processes. These products will generally have a restricted boiling range corresponding to the distillation range used in manufacturing. The #2 fuel oils are an example of this. #6 fuel oils are higher-boiling, or residual materials; #4 fuel oils are often a blend of #6 and #2 oils (Chromatograms 44 , 46 , 48 , 49). The general shape of the above-background envelope of an oil sample, especially the higher end not subject to evaporation, may reveal whether it is a distillate or a blend of distillates and residues rather than a crude oil. On the other hand, the relative amounts of individual normal paraffins and pristane and phytane present, compared to the unresolved complex mixture, depend on the oils used in manufacture

and is not typical of the product. Some products are "dewaxed" or otherwise treated in ways which remove normal paraffins or saturated compounds; lubricant oil (Chromatogram 51) is an example. We have heard arguments concerning whether a sample could be a #6 fuel oil, in which it was contended that a sample could not be "because #6 has a prominent series of peaks and this does not." While the samples in question undoubtedly differed, the argument is incorrect.

Not all oil products that are referred to by different names are necessarily chemically different in a clear-cut way; for example, the marine diesel and #2 fuel oil (Chromatograms 46 and 47) differ in boiling range, but we do not have enough samples of "marine diesel" to be sure the distinction is always clear. On the other hand, such materials as gasoline, asphalt, and mineral oil have been chemically altered, not merely distilled from crude oil; their chromatograms do not have any strong resemblance to crude oils. Automobile gasoline is quite light and could be analyzed with different conditions than those useful for most other oil products.

While the gas chromatograms of different oils from different fields are relatively constant and fixed by geological considerations, the output of refineries varies with economics and technology. In practice, routine sampling programs in a given area should reveal marked changes and enable water quality managers to take them into account.

PETROLEUM: GEOCHEMISTRY

Petroleum geochemistry provides a framework for understanding the main compositional features of oil, the differences between oils and other naturally occurring hydrocarbons, and the effects of several common weathering processes on oil composition. The question of the origin of oil is vigorously debated; the information presented here is a simplification of widely held theories or well-established facts.

The *ultimate* source of the carbon in oil is living matter, but the components of oil are not simply the individual structures present in living matter, concentrated by natural processes. The central issues of oil geochemistry concern the extent, location, and manner in which nonhydrocarbon matter is transformed to petroleum hydrocarbons, and the extent to which oil is simply a collection of small amounts of materials indigenous to life (by geological processes.) Much more is known about the origin of the fraction of MW below 1,000, and especially the hydrocarbons, than about other constituents.

Plants, sediments and soils, and organisms from many environments have been analyzed for low molecular weight hydrocarbons. The only abundant constituents of oil also found abundantly distributed at the earth's surface are the *normal hydrocarbons*, *pristane*, and some of the singly-branched hydrocarbons. The normal hydrocarbons from C_1-C_{60} are also present in oils; adjacent members are present in similar quantities. This distribution contrasts markedly with that of plants, organisms, clean recent sediments, and also shales, which contain a limited range (mostly C_{15}-C_{35}) of normal hydrocarbons and a strong predominance of odd-numbered over even-numbered

compounds. Also, the total natural hydrocarbon content of Recent sediments is often 10-100 ppm. Thus, the hydrocarbon distribution and, in many cases, also the presence of elevated hydrocarbon levels, can distinguish background hydrocarbons from petroleum contaminants. This and related points have been discussed in detail by Blumer (1972) and Appendix I articles.

While part of the normal hydrocarbons and pristane in oils might arise directly from surface materials, much of the n-hydrocarbon content must arise from other matter by subsurface processes. The factors heat, pressure, catalytic action of clays and other matter and bacteria, operating over geologic time, lead to extensive chemical alterations - *diagenesis* - which destroy many original components and create many new ones. This same exceedingly complex chemistry gives rise to the extremely complex mixture of low-level components - the "unresolved envelope"- of oils. This mixture is not (with rare and doubtful exceptions) found in plants and animals, whose biochemistry directs the synthesis of a relatively few materials with high specificity. As an example of diagenetic alteration one might consider the fate of chlorophyll, an extremely abundant biological molecule. The side chain, phytyl alcohol, would yield phytane if it were reductively removed from chlorophyll by reactions easily accomplished in the laboratory. A different sequence of reactions involving the loss of one carbon atom could also easily yield pristane. These compounds in oil arise in part from plant-produced chlorophyll by a series of reactions as suggested by Figure 3. The relative and absolute amounts of these two substances produced and preserved depends on many factors. These factors vary in a complex manner with source material, age and depth of burial, geology of the matrix, temperature, and subsequent history of oil in the reservoir.

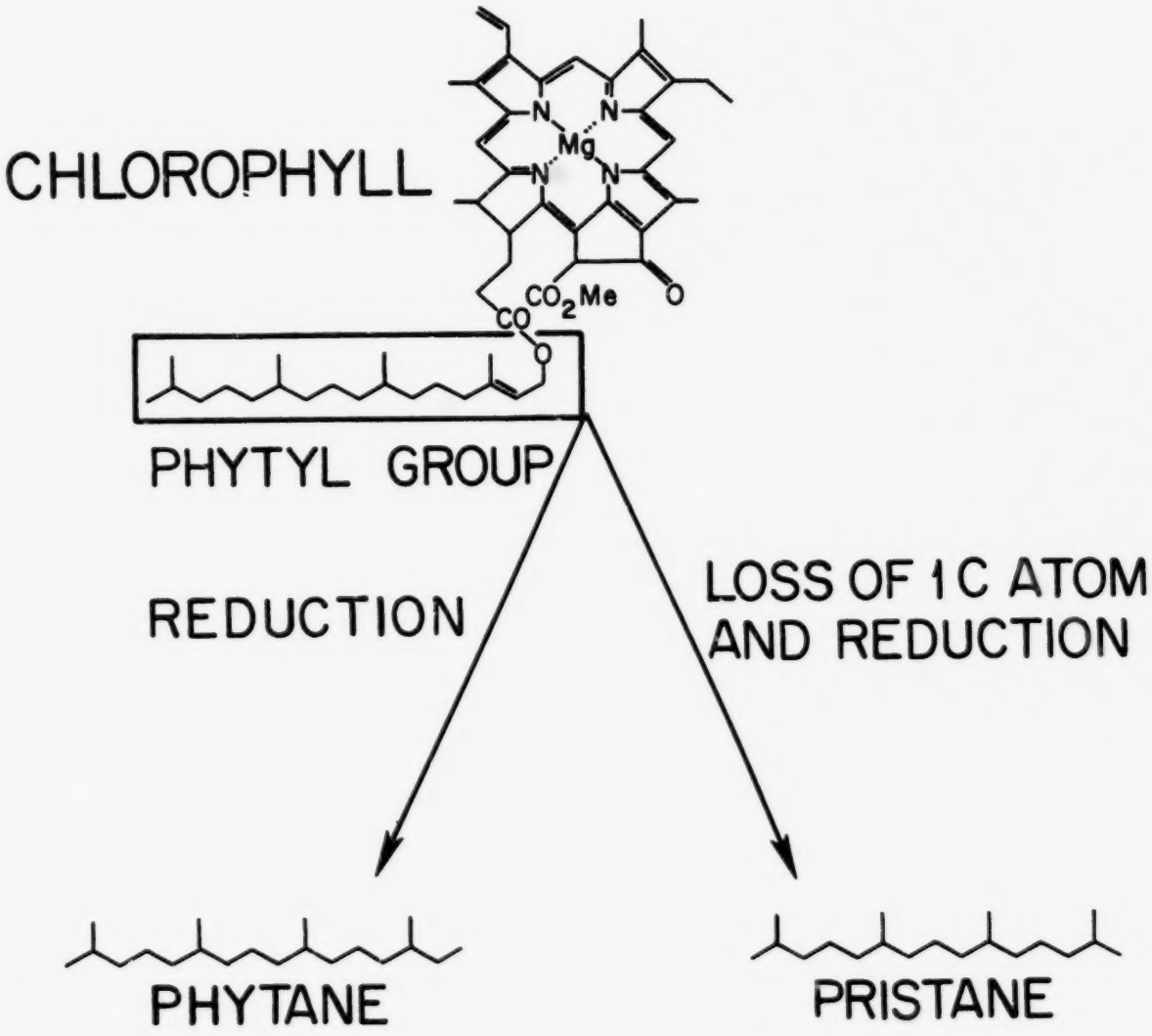

Figure 3. A Geochemical Source of Pristane and Phytane

The *complex mixture of aromatic hydrocarbons* found in petroleum has never been found as a *natural* component in organisms, though some bacteria apparently do synthesize a few simple, unsubstituted or monosubstituted aromatic compounds (Meinschein, 1969). The presence of a complex mixture of *aromatic* hydrocarbons (as shown by column chromatography followed by GC-MS) is probably the best evidence that material is oil-derived. This information may indicate that material is oil-derived even in cases too complex to allow correlation with a source. . Contaminants arising from coal-tar products may interfere; they are rare. Since they are not formed by organisms, complex mixtures of aromatic hydrocarbons can be taken as contaminants if found in organisms.

The geochemistry of petroleum also offers some natural experiments on water-related oil weathering processes. Evaporation, dissolution, and microbial catabolism of some components are the primary processes occurring in weathering; dissolution and microbial catabolism occur in certain types of oil reservoirs. The simplest example is the "open" type of reservoir, in which oil is trapped at a high point in a continuous, permeable stratum bounded by relatively impermeable strata. Oil in such traps is held immobile with respect to groundwater which flows through the strata and past the immobile oil. The oil is "water washed" and the soluble components are selectively removed. The groundwaters may also bring in oxidizing agents (oxygen, sulfate) and bacteria, and so subject the oil to the action of bacteria. Such oils are frequently missing most of the usually abundant low-molecular weight compounds (below C_{10}) and frequently have relatively low contents of the normal hydrocarbons. Sometimes pristane

and phytane are not prominent in these oils. Many detailed experiments and field observations on such oil fields have demonstrated that the loss of low-molecular weight materials is due to their generally higher water solubility, and that the low content of normal hydrocarbons and often of isoprenoids is due to the selective oxidation of these compounds by bacteria. Laboratory experiments show that the normal hydrocarbons are oxidized much more rapidly than isoprenoids and other classes of compounds.

OIL WEATHERING

The primary processes affecting oil in water environments are *evaporation* of the more volatile constituents, *dissolution* of the more soluble components, and preferential *oxidation* by microorganisms of the straight-chain and slightly branched hydrocarbons. These same processes can affect the composition of oils before they are ever produced.

Evaporation is the major and fastest-acting oil alteration process in most spills. Within hours, compounds up to about ten carbons are heavily depleted, and floating oil loses most compounds up to about 13 carbon atoms in a few days. Beached oils may evaporate more rapidly. The oils exposed by EPA (Tables 1 and 2) partially lost compounds up to n-C_{18}, after 1 month floating on water outdoors; n-C_{17} and above were little affected. Evaporation eventually removes most of the components boiling below n-C_{17} or n-C_{18}; this requires roughly a year. A great advantage of the gas chromatographic method is that it separates the sample approximately according to volatility and thus reveals the boiling range.

Laboratory-simulated evaporation of suspected source oils parallels the effect of exposure on spill samples. For samples less than 30 days old, there is no serious alteration of the most useful analytical ratios. Evaporation may make it difficult to classify a sample as a crude which lost light compounds as opposed to an oil product such as #4 fuel oil in which these components were removed in manufacture.

In our experience, short-term (weeks) dissolution has negligible influence on the higher-boiling, non-evaporating fractions of oil used in this correlation method; it probably resembles evaporation enough to be confused with it.

The most erratic weathering factor is microbial alteration of the oil, with preferential oxidation of normal hydrocarbons and, in extreme cases, of the isoprenoids. This oxidation can affect most of the useful analytical ratios used in correlating oils.

For short-term (30 days or less) exposures of oils, there is little evidence that this effect occurs to any important extent. It has been repeatedly shown in the laboratory that under ideal conditions, oil-oxidizing bacteria can alter a sample markedly in a few days; apparently, the marine environment seldom, if ever, provides such ideal conditions. However, several proposed oil-spill control and cleanup methods enhance the rate of such reactions. Seeding with bacteria and nutrients or addition of hay will probably accelerate bacterial action markedly.

Older oil samples may show varying degrees of n-hydrocarbon loss through bacterial oxidation; these samples may be correlated by the use of other parameters.

Thus, evaporation is the main oil-weathering factor on a short-term basis, and can be modelled easily. In theory, bacterial oxidation, because of its potentially erratic nature, might be troublesome, but in fact it seems to be relatively unimportant on a short-term basis in normal situations.

The effect of oil weathering seems to be small on correlation ability for oil-rich samples which are not very old. On the other hand, oil recovered from sediments and/or organisms, or after long periods of exposure, has probably been altered by all weathering processes. It is still possible

in many cases--for example, after two years in the West Falmouth oil spill study--to identify the material as oil and to place constraints on the initial composition. However, correlating ability in the sense demonstrated in Table 1 will often be severely degraded.

A very rough line of division may be drawn between oil-rich water, slicks, sediments and tarballs only a few weeks old, for which the chances of correlation with a suspected source are excellent, and oils which are older or more heavily diluted and admixed with indigenous hydrocarbons in organisms and sediments. In these cases, identification of the specific source will frequently be tenuous (though if the spill source is known previously, a continuous sequence of changes consistent with the known geochemistry of oil may easily be observed). Indigenous hydrocarbons in organisms and in Recent sediments may be distinguished from typical oils. For example, a strong odd over even predominance of n-alkanes and a relatively simple component mixture are typical of indigenous hydrocarbons. Odd/even ratios near 1:1 and the presence of a complex unresolved mixture is typical of petroleum-derived material.

A more severe problem in interpretation may arise if the sediments or organisms already contain oil residues from previous chronic pollution, or if spill and baseline samples are not available. In general, the mixed origin of such materials may be indicated by the sample chemistry, so that useful data for monitoring damage or recovery can be collected; correlation will require careful research.

GAS CHROMATOGRAPHY OF OILS

Before presenting a gas chromatographic method of correlating oils, we outline analytical problems which oils present and solutions that we utilize. These points clarify the purpose of many procedural details and should aid the analyst in modifying or extending the method for related or research purposes.

Most analytical difficulties in gas chromatography arise because *the interaction between the resolving element -- column -- and the sample is very strong*. The more typical resolving element -- prism, grating, electromagnetic field gradient -- never contacts the sample and is only indirectly affected by it. In contrast, the sample passes through the gas chromatographic column and is very likely to modify it irreversibly to some extent.

Oils present particularly difficult samples because of their wide boiling range and content of polar as well as nonpolar materials; furthermore, relatively high resolution is required to obtain the necessary separations. The restraints imposed by higher resolution requirements increase the difficulty of preventing sample modification of the column.

There are two basic approaches to this problem: to pre-fractionate the samples into simpler and cleaner materials for analysis, or to analyze minimally treated samples in a system carefully optimized to provide reliable operation under the adverse conditions presented by oil samples.

We have chosen the second approach because it requires much less operator time and skill and allows the factors which introduce variability to be more tightly specified. The first approach -- pre-fractionation -- can yield more information in the hands of a skilled and experienced chemist,

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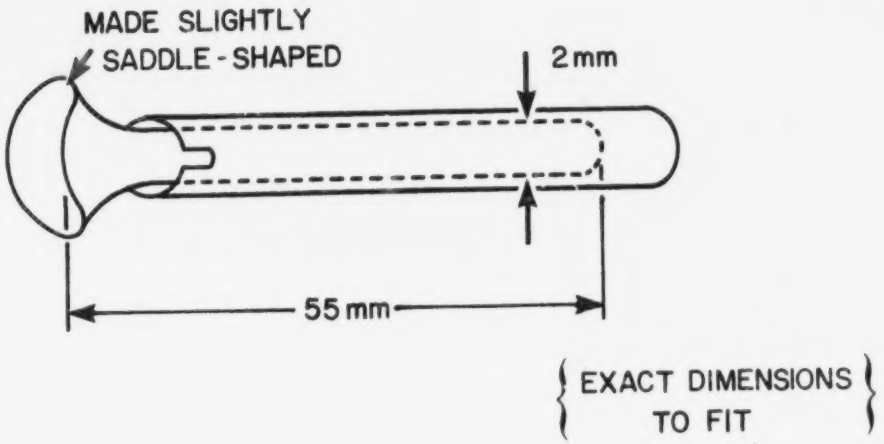

GLASS-LINED INJECTOR ASSEMBLY

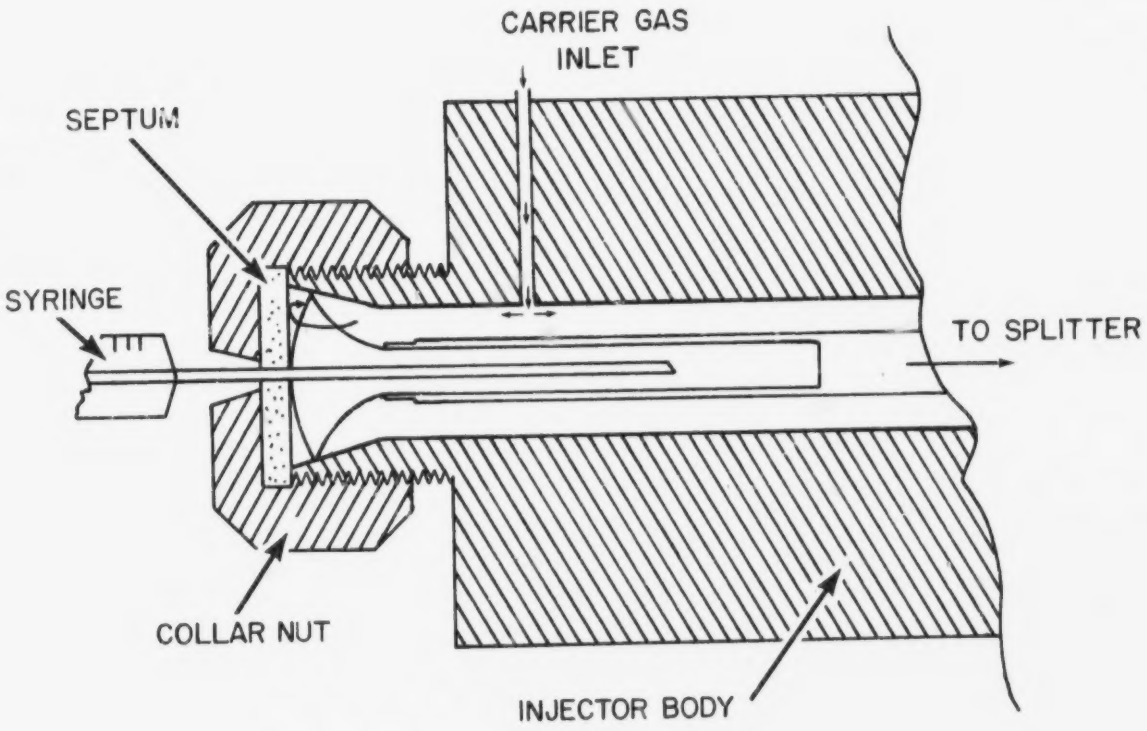

Figure 4. Glass-Lined Capillary Injector

but can actually increase variability if improperly used. It is unsuited for large-scale programs or routine work, though it should be applied to specific problems of particular interest. The addition of a pre-treatment step is compatible with the method presented.

For routine correlation, the method accepts oil samples as dilute solutions in purified carbon disulfide. These solutions are easily prepared from oils and oil-rich water and sediments. Spill-source correlation of oils extracted from dilute water samples, from sediments, and from organisms requires:more elaborate sample preparation procedures, analysis of comparable unpolluted samples for background determination,and careful interpretation; many laboratories may consider these to be nonroutine applications. These situations grade into research problems.

These carbon disulfide solutions of oil sample are introduced by syringe into the capillary injection port of the instrument. Since the samples will often have a non-volatile residue, we have devised a removable glass liner for capillary injection ports much like those conventionally utilized with nonsplitting inlets (Figure 4). The method utilizes the "trapping" property of a cold column to eliminate the necessity of using the conventional, high injection port temperatures (300-350^{o} C) often used for oils; this eliminates column-degrading residue pyrolysis and septum "bleed" while reproducibly introducing the sample onto the column. Both pyrolysis and bleed load the column with high-boiling materials, which shorten column lifetime and increase variability. We utilize an

injector temperature of 200° C, since in programmed temperature GC true "flash" vaporization of high-boiling materials is unnecessary for relatively late-eluting materials; the early-eluting compounds are flashed into the column. While the cold column attached to a hot injector traps all high-boilers on its front end, it can also accumulate large amounts of septum bleed or exhibit "memory effects" on the next program. A bakeout procedure after periods of disuse eliminates this problem.

The injected sample is split to an optimal size and the sample feeds to the "SCOT" (support-coated open tubular) column coated with the nonpolar liquid silicone OV-101. This choice of column is, in our opinion, close to optimum for the applications discussed. Polar packed columns can separate pristane and n-C_{17}, and phytane and n-C_{18} well, but do not have the temperature stability to handle higher-boiling materials. Non-polar packed columns tend not to separate the pristane/C_{17} and phytane/ C_{18} pairs well unless they are long and are operated at high temperatures. For packed columns, 6 - 10' low-loaded Apiezon L is close to an optimum choice.

Capillary columns with various coatings render excellent separations of the peaks of interest, but are sensitive to deterioration or destruction by addition of high-boiling materials. They have a short lifetime unless sample pre-treatment to eliminate residues is incorporated.

Support-coated capillary columns offer a compromise between packed-column durability, and wall-coated capillary resolution and low retentivity. The length and openness are capillary-like, but the support-coated wall, with its much higher load of

stationary phase, increases capacity and durability. The flow resistance is low and reproducible from column to column; variations in this factor restrict comparisons of results from different packed columns.

The particular coating chosen for the SCOT column, -- the silicone OV-101 -- has several advantages. OV-101 provides good separation of both the C_{17}/pristane and especially of the C_{18}/phytane pair. The separation is complete enough that changes in resolution (peak sharpness) hardly effect most ratios, facilitating comparison and correlation. Use of helium as carrier gas is essential for this good resolution performance with SCOT columns. The second advantage of OV-101 substrate is its high thermal stability. The column is always operated at least 75^{o} C below its maximum recommended temperature, prolonging column life and minimizing bleed of silicones into the detector and subsequent fouling.

We have tested "SCOT" columns of Apiezon L and of "Dexsil 300" (Chromatogram 52) , but neither separates n-C_{18} from phytane well. In our experience, substantial further improvement over SCOT OV-101 column performance would require capillary columns and preliminary separations to protect them.

We have not found special innovations in detection (flame ionization is used) or recording to be necessary; though these components must function properly, and the temperature programmer of the GC must be reproducible and calibrated.

The GC instrumentation summarized above is capable of producing chromatograms of oils that are highly useful in oil correlation. To ensure that

the entire analytical system is performing correctly, to provide comparability of the peak height ratios given by a particular column/instrument combination, and to measure the variability of the parameters utilized in correlation, the analyst runs frequent blank determinations and chromatograms of a standard oil sample; a #2 fuel oil stabilized against chemical decomposition by removal of polar impurities has been chosen. *Frequent analysis of the standard is an extremely important part of the procedure.* It permits the analyst to detect and correct for small variations in column performance, to detect any increase in variability of results (which suggests some fault of the equipment or technique) and to estimate relative standard deviations which apply to other oil samples. We have shown that the variability of a given ratio determined on the #2 fuel oil is applicable to crude oil analyses also. Estimating the variability of ratios is important in interpreting the significance of proposed correlations.

Table 6 presents the relative standard deviations (RSD) of several analytical ratios for a #2 fuel oil standard. The analyses span considerable use periods and three different columns in three different instruments (of the same make and model). The relative standard deviations are largely in the range 1.6 - 3.5%, even though new procedures were being developed; clearly deterioration of the columns is a minor factor. Note that only three of the first four ratios are independent, as four independent quantities are ratioed. Variability related to fractionation and temperature effects should make the largest error contribution to the 17/18 and pristane/phytane ratios; shifts in elution temperatures or

TABLE 6

LONG-TERM VARIABILITY OF ANALYTICAL

RATIOS OF #2 FUEL OIL STANDARD

CONDITIONS			% RELATIVE STANDARD DEVIATIONS OF RATIOS[a]					
Column	Total # of runs	Injection #[b]	Pris/Phy	C_{17}/Pris	C_{18}/Phy	C_{17}/C_{18}	C_{17}/BG	C_{17}/17-Pr valley
216	13	121 - 136	2.15	3.11	2.85	2.56	4.52	
218	11	33 - 175	2.44	2.30	1.80	2.86	2.98	3.14
219	11	34 - 207	3.43	2.60	1.59	1.56	6.21	7.38

a Ratios measured as defined in Figure 1 and related discussion

b Each sample injection is numbered; tabulated analyses of standards occurred during the interval given.

TABLE 7

VARIABILITY OF CONSECUTIVE ANALYSES OF CRUDE OILS FOR SEVERAL SCOT OV-101 COLUMNS

CONDITIONS			% RELATIVE STANDARD DEVIATION OF RATIOS[a]				
Column[b]	# runs	Crude Oil	Pris/Phy	C_{17}/Pris	C_{18}/Phy	C_{17}/C_{18}	C_{17}/BG
216[c]	6	TJ lt. (crude)	3.94	3.00	2.65	4.16	9.59
218	6	Agha Jari	2.20	0.70	1.07	2.87	3.41
218	6	Agha Jari	2.10	0.82	0.91	1.75	1.85
219	6	Agha Jari	5.83[d]	1.30	1.72	6.10[d]	2.68

a. Ratios measured as defined in Figure 1 and related discussion.

b. Columns used had been utilized previously for 80 - 160 analyses.

c. After period of disuse; variability may be caused in part by "atrophy effect".

d. See text. Variability decreased after injection port cleaning.

TABLE 8

INTERCOLUMN COMPARISON OF RATIO MEANS

CONDITIONS			SIGNAL INTENSITY RATIOS[a]				
Column #	# Runs Averaged	Sample	Pris/Phy	C_{17}/Pris	C_{18}/Phy	C_{17}/C_{18}	C_{17}/BG
218[c]	11	#2 Fuel oil[b]	1.81	1.41	1.90	1.34	4.36
219[c]	11	#2 Fuel oil[b]	1.88	1.41	1.90	1.40	4.34
216[d]	13	#2 Fuel oil[b]	1.83	1.29	1.65	1.44	3.42
218[d]	6	Agha Jari	0.93	1.97	1.59	1.17	5.56
219[d]	6	Agha Jari	0.89	1.93	1.55	1.11	5.49

a Ratios measured as defined in Figure 1 and discussed in text.

b Standard sample.

c Runs made during period of ~150 injections.

d Consecutive analyses.

resolution changes affect primarily the 17/pristane and 18/phytane ratios; the C_{17}/background ratio should be particularly sensitive to resolution changes. Apparently a conglomeration of factors contribute to variability, as ratios show similar RSD's.

Table 7 shows the RSD's obtained with three columns for several samples when analyzed repeatedly in a short time period. With a few exceptions (caused by unusual sample type or evident instrument malfunction) these RSD values are about half those found over column lifetimes; the better performance of the second and third columns is clear. The high variability of ratios involving compounds of different boiling points for column 219 suggested vaporization-related problems: cleaning the injector led to an immediate decrease in the variability of these ratios.

Thus, the properly operating system yields RSD values of the order of 0.7 - 3% over short time periods, and less than twice this level over longer time periods. Failure to achieve this level of reproducibility suggests deficiencies in columns, instrumentation or technique.

The comparisons above illustrate the reproducibility of results from a single column; Table 8 compares the absolute values of ratios obtained for the same sample on different instrument/column combinations. The three tested columns are clearly quite similar; for several ratios, the difference among different columns are not over one standard deviation for the repeated analyses of the same sample at the same time (See Table 8). The greatest ratio differences are for those affected by resolving power. Column #216 was definitely somewhat less efficient. The smaller ratio differences are often unimportant; larger ones are compensated by a correction factor to

make results intercomparable.

These RSD and ratio comparisons illustrate the degree of reproducibility obtainable. Some of the variability in these ratios has been traced to sample size factors which can be controlled. Comparisons of similar-sized samples have smaller ratio variabilities. The effect of sample size on various signal intensity ratios is shown in Figure 5; such plots may differ from column to column and to a small extent with sample type.

The additional effort required to maintain records of column behavior with a standard sample is well worthwhile. Analyzing a standard several times consecutively is a powerful check on procedure, as increases in variability or change in absolute ratios are revealed, and indicate necessary corrective action. Since the relative standard deviations obtained from one sample type seem to be applicable to other samples, estimates of the significance levels of observed ratio values can be made even for spill samples analyzed once. For example, if pairwise comparison of data were necessary for all samples, the data of Table 1 would require over 1,000 GC analyses, surely expending at least one column in the process. In fact, duplicate analyses of all samples and accompanying #2 fuel oil standards and blanks enabled us to make most comparisons with 120 analyses; only a few very similar pairs were then reanalyzed in an "ABAB" format; this procedure yielded the data of Table 1.

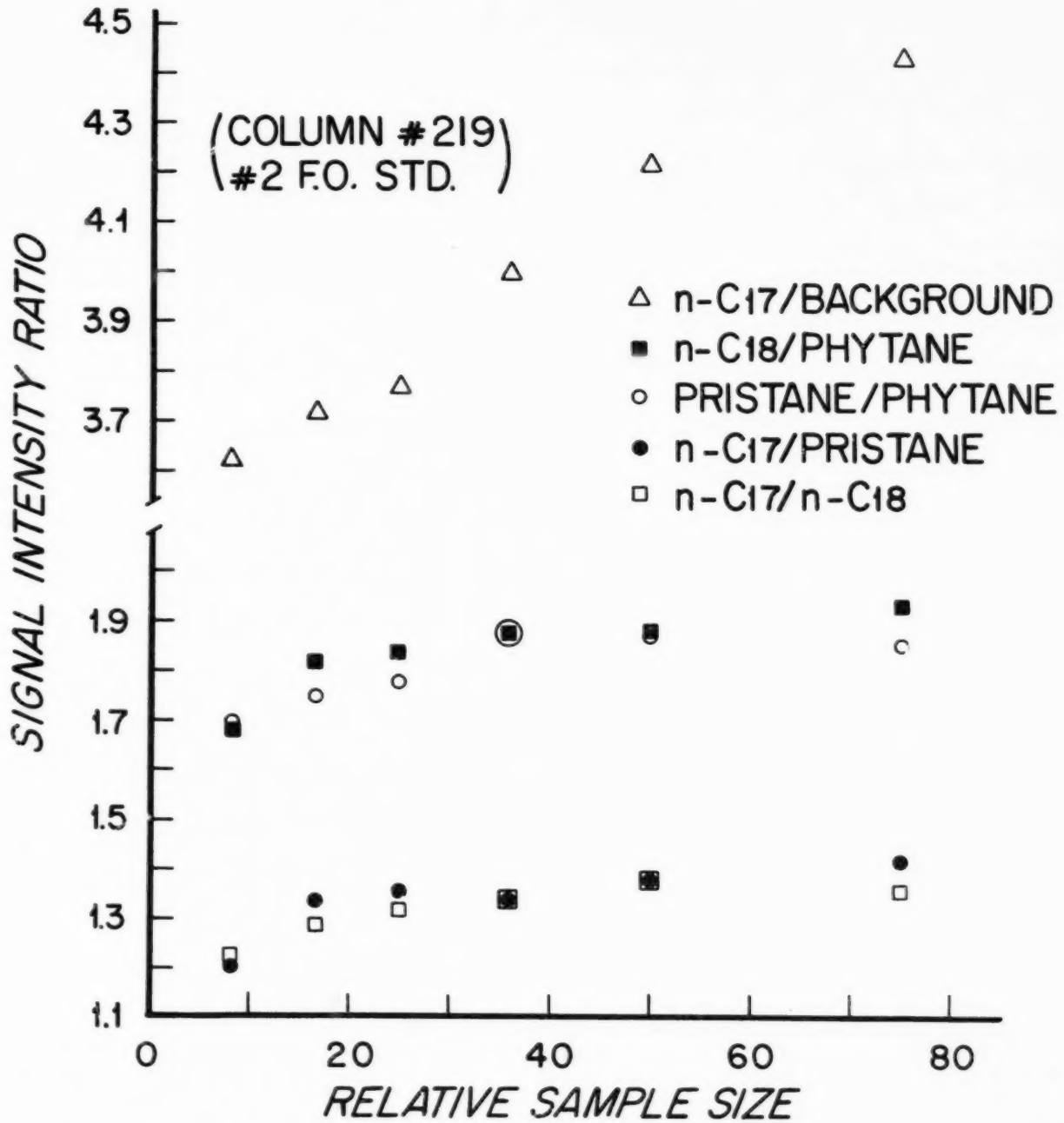

Figure 5. Relation of Ratio Values to Sample Size

GAS CHROMATOGRAPHIC METHOD FOR OIL ANALYSIS

1. Samples and Sample Storage

These operations should obtain an adequate, documented, uncontaminated representative sample preserved without alteration or loss of components.

Source oils: These samples are generally oil without other admixed material and are obtained from tanks, pipelines, holds, etc. The sampling location and method (pipeline withdrawal, bucket sample, sample thief, etc.) should be recorded. The sample *must* represent the material of interest; for example, pipeline "T" connections, etc., may contain previously handled materials and require flushing; some vessels carry different cargos in different holds. The analyst or his representative should be present at the sampling operation; a photograph of the sampling location is also highly desirable. Often samples of a pint or more are easily obtained; a few drops are adequate but an ounce to a pint is desirable. Inhomogeneity (for example, possible layering in tanks) requires obtaining several subsamples from different locations. Glass containers or metal cans with tight closures should be rinsed with distilled methanol and pentane and dried; they should have tight-sealing caps of Teflon or oil-resistant plastic. Rubber or waxed paper cap liners are not satisfactory. Each sample should be accompanied by an adequate description including location, sampler, method, and precise element (tank, line, etc.) sampled. Any spill control chemicals utilized should be sampled in this same manner.

Samples should be refrigerated if held more than a few days after collection. Sealed containers nearly full of oil and devoid of air layers are stable

for months or longer. A most important point in source sampling is to *obtain samples of all the possible sources* in the area as soon as possible after the spill. One cannot correlate an oil with a sample which is not available.

Oil in Water: Only micrograms of oil are required for analysis; however, samples must be representative of the contaminant. Larger samples (several ml to a pint) are more likely to be representative and also provide material for further analyses. Substantial discharges should be subsampled at a variety of locations. Similar materials should be sampled from nearby unaffected areas to serve as controls.

So long as they do not contaminate, several methods of obtaining oil-rich samples are acceptable. The apparatus must be free of oil-soluble components. Electronics grade fluorocarbons are good solvents and are nontoxic and nonflammable. They can be used safely in the field to clean emergency sampling equipment and containers. Oil has been collected by dip-netting, by picking up lumps, by slowly submerging wide-mouthed jars or buckets, by scooping along the edge of "booms", by immersing a mop in a slick and wringing it out, and by collecting oil-soaked straw or other cleanup agents. Methods which use clean materials and obtain relatively oil-rich samples are most satisfactory; dilute samples should be accompanied by blank samples if apparatus of uncertain reliability is used. A major advantage of collecting concentrated oil samples is that rapid alteration by microbiological growth is much less likely than

for dilute oil in water or oil soaked in nutrient-rich organic matter such as hay . If bacterial seeding of oil spills is ever adopted, sampling procedures will require modification to preserve samples more effectively. Samples should be prepared for analysis or frozen as soon as possible.

Very dilute oil in water samples may be collected by using classical "water-catching" methods of oceanography and hydrology. These samplings will require careful apparatus preparation, avoidance of ship contamination (as from bilgewater and lubricants) and hydrographic wire lubricants. Immediate sample extraction or preservation to inhibit bacterial growth will also be necessary. Such dilute samples are research problems or sophisticated monitoring tasks, and are not suitable for routine analysis.

Oil in Sediments and Organisms: Shallow water sediments and organisms can be sampled by simple jar collection; in deeper water, a pre-cleaned unlubricated dredge or grab sampler may be used. Fishnets and other ship-deck equipment are often oil soaked and unsuitable for use without cleaning and obtaining a blank sample. The surface 1 - 2" layer of sediments obtained by grab sampler (Van Veen type) is separated by "spooning" this layer into a pint mason jar. The grab used should protect the sample from excessive water washing or homogenation. Short (1 - 2') sediment cores obtained with an oil-free apparatus are also very useful samples. Sampling locations should be chosen after considering the physical and biological character of possible locations.

Organisms and sediments should both be frozen as soon as possible after collection to prevent extensive bacterial growth. Control areas or organism populations should be established and sampled in nearby similar locations to provide comparison or baseline data. Relatively large commercially valuable organisms are often selected for analysis. Bottom-dwelling (benthic) organisms which are unable to escape an area are more likely than fish to show clear contamination. Sedentary organisms are especially suitable for study, as the same population may be repeatedly subsampled over a period of time.

Shellfish are particularly suitable for analysis. If an "effects" study is contemplated, sampled organisms should be selected in consultation with biologists. Samples should be taxonomically identified before being destroyed by analysis, or individuals may be preserved by classical biological methods.

Sediments in locations not known to have been affected by major oil spills have been found to contain high levels of oil-derived hydrocarbons in some locations; these presumably arise from chronic discharges. It is desirable that "baseline" organisms and sediment samples be taken and analyzed in locations subject to water quality control management, as these "dribbling" discharges may have effects as large as those from discrete spills in some locations.

2. Sample Preparation

The purpose of this step is to prepare a carbon disulfide solution

(ca. 5-10% wt/vol) of a portion of sample for GC analysis without loss of the components utilized in correlation or introduction of contaminants. The concentration need not be known exactly. For oils and relatively oil-rich samples, these solutions are easily prepared as outlined below; for progressively more dilute samples admixed with larger amounts of matrix and with naturally occurring hydrocarbons, more elaborate procedures and controls become necessary. Treatments to remove interfering materials are required, and the ability to discriminate among alternative source oils diminishes. However, the presence of oil contamination can be established under conditions which preclude correlation with a specific source.

As a rule of thumb, samples of straight oils, oil lumps and tarballs, oily water, oil emulsions, and oiled surfaces (rock, shells, coarse sand, wood, cleanup straw, brooms, etc.) are easily processed; the soluble portion of these samples is often contaminant oil, with little or no admixed natural hydrocarbon background. These samples are suitable for routine analysis and attempted correlation. Extraction of sediments and organisms for oil requires more skill, time, and precautions; correlating ability depends on the individual situation. These materials can be monitored, often at ppm levels, for the presence of oil or oil products.

Recently, concerted efforts to devise and standardize procedures for extracting and detecting low levels of oil in organisms and in sediments have been made; Appendix 3 is a preliminary summary of these efforts,

made under the sponsorship of the International Decade for Ocean Exploration (IDOE) at Woods Hole Oceanographic Institution and elsewhere; more detailed reports will be available shortly. These should be consulted. The GC columns employed in this study are capable of analyzing the materials obtained by these sample-preparation procedures.

Oil-rich Samples: Oil samples or samples which are predominantly oil (such as "tarballs", very oily sand, thick deposits removed from surfaces such as rocks, shells, pilings) are prepared by dissolution in carbon disulfide and separation of water and particles by centrifugation. The thawed sample is shaken, stirred, or otherwise homogenized, and a portion (containing an estimated 50-100 mg of oil) is placed in the bottom of a centrifuge tube equipped to accept Teflon-lined screw caps. Thin oils can be transferred by dropper or capillary; thicker ones on a rod. All carbon disulfide and other solvents used are reagent grade, redistilled in all-glass apparatus (CAUTION: CS_2 *has a flash point -22° C*) and shown by 100x evaporative concentration and GC analysis of the residue to be free of interfering materials. Sufficient carbon disulfide is added to result in an estimated 5-10% solution (for pure oils -- suspected sources -- it is convenient to weigh oil and measure CS_2 volumetrically so the concentration is accurately known); heavier and darker oils generally require 10% solutions; this is not critical. The use of 50-100 mg of oil and 1.00 ml carbon disulfide in a 13-ml centrifuge tube which can be closed with a Teflon-lined screw cap is recommended. The sample is agitated for several minutes, as with a "Vibromixer". All the components of interest

in oils are soluble in CS_2, and all but the thickest oils dissolve rapidly; thick oils should be smeared as a thin film on the tube walls. Very thick samples may be warmed in these capped tubes to about 50° C and agitated. The sample is then centrifuged to remove insoluble particles and water globules. Removing about half of the solution from mid-depth with a syringe equipped with a long needle leaves behind supernatant bulk water and solid residues. The resultant solutions are stable for months if protected from light and evaporation by storing in glass vials with Teflon-lined caps in the dark. If possible, store samples in this manner rather than as frozen oils, which may precipitate waxy deposits difficult to redissolve.

Oily Water, Sand, Straw, Etc.: Often such samples can be treated by the procedure given above after a simple step, such as scraping the oil coating off shells; a common sample is water of 1-10% oil content with some solid material present. Centrifuging such samples for 20' at 2,400 g (clinical type centrifuge) often yields well-defined surface or sunken layers allowing the removal of water. The oil residue is treated as an oil-rich sample; if oil collects at both the top and the bottom, both portions should be recovered by pipetting or syringing out the water.

More dilute oily water -- 1:1,000 or more -- is extracted with several small volumes of carbon disulfide in a separatory funnel.

If solutions are too dilute (estimated by trial GC analysis or by evaporating an aliquot and weighing the involatile residue) they may be concentrated on a rotary evaporator by controlling the suction at above 100

mm Hg; full mechanical pump vacuum is applied for 30 seconds at the end of the evaporation to yield nearly solvent-free material. This method removes most of the material boiling below n-C_{12} but hardly affects components boiling above n-C_{15}.

Sediments and Organisms: These samples require more elaborate cleanup procedures and parallel determinations of the indigenous background of pre-spill samples. All apparatus and solvents must be purified before use. Pentane, benzene, and methanol are distilled in all-glass equipment through a packed column and checked for residue (see CS_2 procedure). Granular anhydrous sodium sulfate, boiling stones, paper Soxhlet thimbles, and filter paper such as Whatman #1 are purified by 24-hour Soxhlet extraction with 1:1 benzene-methanol, air dried in a clean hood, and oven-dried at 110° C. The adsorbents silica gel (equivalent to Davison chemical Grade 922, 200-325 mesh) and alumina (equivalent to Harshaw Chemical Company, AL 0102P) are Soxhlet extracted with 1:1 benzene-methanol, air-dried, activated (silica gel, 120° C; alumina, 250° C) and then deactivated with water (silica gel, 5% w/w; alumina, 6% w/w) by thorough mixing and 24-hour standing. Prepared adsorbents may be stored in tightly stoppered all-glass bottles. Adsorbent activities and consequently chromatographic results vary from batch to batch of adsorbent; chromatographic procedures, though generally applicable, are not entirely standard and should be checked for each new sample type and reagent batch.

The thawed sediment or organism sample (50-100 g is convenient) is loaded into a Soxhlet thimble (not more than 3/4 full). If sample crushing or cutting is necessary to reduce the size, clean, solvent-insoluble

surfaces and apparatus must be used; for organisms, specify the material taken (e.g., "whole organism", "shucked shellfish" (with or without internal fluid), "muscle only"). The thimble is set in the extractor body on a large glass stopper or plug to facilitate good drainage, and extracted with 1:1 benzene-methanol (about 4-5x extractor body volume). The solvent level in the boiling flask should never fall so low that the dry flask wall is heated directly. The extracting solvent is changed after several hours; if heavy foaming occurs (as with some biological materials) an earlier change or two changes may be required. The total extraction time should be at least 24 hours.

Next, the extracted material is prepared as a solvent-free lipid fraction and weighed prior to column chromatography. If the organic extract contains only small amounts of particulate matter and if a small test portion of extract does not emulsify on diluting 10:1 with distilled water or with half-saturated sodium chloride solution (previously extracted with pentane to remove contaminants), the procedure is straightforward. The entire extract is then decanted from solids or filtered through paper, diluted, extracted five times with pentane (in total about half the aqueous volume); the combined pentane layers are dried over sodium sulfate and filtered. The flask and drying agent are rinsed with fresh pentane and the pentane solution is evaporated; the residue of extracted material is weighed.

However, if the benzene-methanol extract contains much particulate matter, the extract is centrifuged and the solid portions are resuspended in pentane, agitated, recentrifuged, and the pentane layer drawn off and added to pentane used to extract the diluted benzene-methanol supernatant layers.

If the water dilution test shows the formation of emulsions which do not break in ca 30 minutes (some biologically-derived samples) difficulties may be encountered. If a large-capacity centrifuge is available to treat the entire diluted extract, the trial portion is tested to see if high-speed centrifugation will break the emulsion; if so, the dilution procedure followed by centrifuging may be used. Otherwise, the benzene-methanol extracts may be repeatedly (5x) treated with relatively small volumes of water or salt solution and stirred slowly but thoroughly to minimize emulsification. All five water extracts are saved and back-extracted with pentane.

Acidification of extracts to pH 2 may minimize emulsification, especially for samples rich in free fatty acids. Organisms extracts may also be treated by saponification with methanolic KOH and subsequent dilution with water and extraction of the nonsaponificable materials--including petroluum hydrocarbons--into pentane. See Appendix 3 and references therein for further information.

Once the initial phase separation and extraction of material is accomplished by any of the above methods, the sample is dried, concentrated, and weighed.

All sediment and organism-derived extracts must be purified before gas chromatography by column chromatography. Sediment extracts also require removal of elementai sulfur by reaction with powdered copper. Samples (the entire extract or 0.5 g, whichever is smaller) are chromatographed by placing on a column in a minimum volume of pentane and eluting the column with four column volumes of n-pentane. The columns

are prepared by wet-packing at least 60x the sample weight of silica gel in pentane, and covering this with at least an additional 40x the sample weight of alumina; both adsorbents are purified and deactivated as previously described. The chromatographic tube should be chosen such that the packed bed height is 15-25 times the internal diameter; in practice, the available columns often determine an optimum adsorbent weight and the maximum acceptable sample weight. These water-deactivated dual-absorbent columns have high capacity for high-molecular weight non-hydrocarbons, and do not tend to convert sensitive nonhydrocarbons to artifact hydrocarbons. These columns have been found to give 99±1% recovery of fuel oil hydrocarbons, a carbon number range which covers the most commonly utilized peaks for oil correlation.

The suitability of the column for removal of nonhydrocarbons is checked for each batch of adsorbents and each new sample type. A thick-film infrared spectrum on NaCl plates of the concentrated column effluent (micro-apparatus may be required) should show no -OH, C=O, or cis- and trans- di-substituted double bond absorption. Presence of these bands establishes that polar materials have broken through and requires that column parameters be checked and samples be rechromatographed.

The concentrated column effluent is weighed and the material in the Soxhlet cup is air-dried for several days with occasional turning, and weighed. Discard adsorbents after a single use.

Carbon disulfide solutions of the concentrated column effluent from organism extracts are analyzed by gas chromatography. The hydrocarbon content may be calculated as the column effluent weight divided by the dry weight of the extracted residue; however, the residue is not necessarily

all detected by GC analysis, so that the gas chromatogram cannot be assumed to represent all of the injected material.

Sediment-derived samples require removal of elemental and reactive sulfur, which comes through column chromatography, before GC analysis. Often the sulfur content of sediments is quite high, and this step cannot be neglected. Electrolytic copper dust (twenty-five times the weight of the extract to be treated) is covered with concentrated hydrochloric acid and stirred on a sintered-glass funnel. The acid is filtered off without drawing large amounts of air through the copper. This washing may also be performed in a centrifuge tube. The cleaned, bright copper is washed with successive portions of water (2x), methanol (3x), benzene (1x) and pentane (1x), draining well after each washing. The Cu powder is transferred in pentane to a chromatographic column. The packed Cu bed should be at least 20 times as long as the column internal diameter. The column is washed with several bed volumes of pentane, and the sample is slowly percolated through the column and rinsed with 4 bed volumes of pentane. The removal of sulfur is accompanied by blackening of the upper part of the column; if more than the upper third of the column blackens, the sample should be treated again. If no blackening occurs, the column should be tested with a small amount of pentane solution of elemental sulfur. If no blackening then occurs, the column was inactive and the sample must be retreated. These columns should be freshly prepared; if thoroughly rinsed, they may be used for several samples on the same day.

In comparing the hydrocarbon distribution of polluted sediments and shellfish with that of suspected source oils, it is necessary to submit all the likely source oils to the chromatographic procedure before spill-

source correlation.

3. Preparation of Gas Chromatograph and Column

Varian model 1440 gas chromatographs equipped with capillary injector kit have been utilized exclusively in development of this method. While other gas chromatographs can undoubtedly be utilized, we are unable to specify necessary modifications or problems they may present; in particular, capillary injection design varies widely.

Set-up and checkout procedures specified in the instrument manual should be performed. Additionally, the indicating pyrometers' and temperature programmer settings of various gas chromatographs require checking. An accurate (± 1° C) calibrated thermocouple or other temperature element should be used to measure the temperature in the injection port, the oven at various temperatures, and the injector base. A thermocouple in a clean glass capillary tube sealed at one end, should be used. The instrument pyrometer should be calibrated and the isothermal and temperature-programmed limits checked and adjusted (see Instrument Manual). The accuracy of the program rate at 6°/min (similar rates of 5 or 7.5°/min are acceptable; most instruments can easily be modified to 6°/min) is checked by timing and observing the dial rotation. We have experienced uneven rotation of the mechanical slip-clutch which connects the step motor to the program rate potentiometer in several of these

instruments.

An easily replaced borosilicate glass liner is installed in the capillary injector. Fig. 4 illustrates the shape and position of the liner in the injector. The tubing is 55 mm long, 2 mm "Pyrex" tubing; the end flare illustrated must be large enough to guide the needle through the tube, but not so large as to form a seal against the injection port's flare or the septum. The central slotted tube of the injector may be irregular in form or have a burred edge; the tubing can be rounded and deburred by gentle manipulation with a small circular file. There is no danger of injuring the split orifices by this procedure; it is not accessible from the injection port.

The gas chromatograph should be connected according to manufacturer's specifications to a 1-mv full-scale recorder of 7-10" chart width with better than 1 sec response time and small deadband. We have utilized Leeds and Northrup Model H recorders. If chromatograms are to be photographed as evidence, choose an ink which photographs well (dark red). We strongly recommend the uniform use of a chart speed of ½" per minute, with signal increasing with pen movement to the right. The resulting chromatograms read from right to left, "backwards" from the usual convention; they are not easily confused with traces run conventionally. Comparisons of tabulated data do not depend on recorder speed or format, but detailed visual comparison is much easier if traces are all of the

same signal polarity, temperature program, and chart speed.

The columns are support-coated open tubular SCOT columns obtained from the Perkin-Elmer Corporation, Column Coating Facility, Norwalk, Connecticut, by ordering "50 foot x 0.02 in. SCOT columns coated with OV-101, to fit Model (instrument used) GC; coated without use of wetting agents, and rating 25,000 effective plates on the Perkin-Elmer hydrocarbon standard." Two slotted male SS 1/16 in. nuts, Perkin-Elmer #009-1593, are required to connect each column to a Varian 1440 with capillary injector. The three columns described in this study were supplied within six weeks of ordering them; the two ordered simultaneously were most similar. Though a single column has a fairly long lifetime (apparently at least 200 oil analyses), we recommend ordering them in pairs. The *feasibility of some central group obtaining larger batches and conditioning and intercomparing them should be considered.*

The columns have been pre-conditioned at 160° by the manufacturer, and are further conditioned *with the column disconnected from the detector* as in Table 9. This conditioning schedule yields columns which still change bleed characteristics fairly rapidly over the first 30 or so injections. The initial period may be used to familiarize the operator with the method. T-programmed column stationary phases become conditioned differently during repeated programming than isothermally; we recommend periods of isothermal operation at 275° interrupted with repeated programming sequences for any further conditioning desired.

TABLE 9

CONDITIONING OF SCOT OV-101 COLUMNS[a]

DURATION	TEMPERATURE	CARRIER GAS (He) FLOW
2 hours	220° C	6 ml/min
2 hours	250° C	6 ml/min
Several standard #2 FO injections with program 75-250° C @ 6°/min		
16 hours	225° C	
30 minutes	250° C	
Five to ten injections of #2 FO	Program to 250° C	Adjusted[b] for CH_4 RT = 44 sec at 75° C
1 hour minimum	275° C	Adjusted for CH_4 RT = 44 sec at 75°
Several[c]	75° - 275° C at 6°/min	Adjusted for CH_4 RT = 44 sec at 75°

a Column not connected to detector

b See daily operating procedures

c Column usable but further operation at 275° C increases baseline stability

TABLE 10

GC OPERATING CONDITIONS FOR OIL ANALYSIS

Injector:	195-210° C; Use silicone septum and glass-liner. Helium flow 50 ml/min, measured at split exit.
Column and Oven:	Temperature programmed from 75° to 275° at 6°/min, with isothermal operation at 275° up to 35 min. He carrier gas; flow rate adjusted to a methane retention time (MRT) of 44 ± 0.5 sec at 75° C.
Detector:	Flame ionization operated at 300 ± 10° C with 10 ml/min He makeup gas and optimized compressed air (not pumped lab air) and hydrogen flows.
Electrometer:	Sensitivity 1 x 10^{-10} amps/mv.
Injection:	0.5 - 5.0 microliters of CS_2 solution by syringe.
Recorder:	1 mv, 7" or greater span, 1-sec response; Operated at 0.5 "/min chart speed with response positive left -to- right

Table 10 summarizes the operating parameters utilized for analysis; adjust the instrument settings accordingly. The methane retention time (MRT) is measured by stopwatch as the time lag between injection of about 1 µl of methane (burner gas stored in a septum-covered flask) and the initial recorder pen rise. This provides an accurate measurement of carrier gas flow rate without disconnecting the column or using inaccurate flowmeters often supplied with gas chromatographs. If a rapid, smooth injection is not made, methane will exit in multiple peaks corresponding to a series of closely spaced mini-injections ; if so, repeat the procedure.

Good resolution is obtained with SCOT OV-101 columns in modern GC instruments without injection splitters, if the detector dead volume is small and is swept with make-up gas. Samples (0.05 - 0.5 ul) may be introduced directly by 1 µl syringe (these require careful cleaning) or directly (10 - 20 ug) as films on a glass rod as described by Blumer, 1972. The latter technique distorts the chromatogram in the non-critical (below n-C_{13}) range. Although resolution is adequate with non-splitting conditions, we have not determined the short or long-term reproducibility attainable. The RSD's of ratios of widely separated components may possibly even decrease without the splitter.

The instrument setup is completed by running a series of standard #2 fuel oil analyses until the analytical ratios become essentially constant, and then estimating ratio relative standard deviations of 5-8 runs as illustrated for several columns in Table 7 ; similar RSD values should be

obtained, and if the same standard #2 fuel oil (submitted to EPA, Edison, New Jersey) is used, similar absolute ratios and a similar chromatogram appearance to those in Appendix 2 should be obtained; exact coincidence is not expected.

4. Analyses and Daily Operation

At the beginning of each day and/or series of analyses, the following procedure is followed: the glass tubing liner is changed by unscrewing the septum nut, pulling out the old liner with a bent wire, and inserting a clean untouched one. Return from standby to active condition by turning on air and hydrogen flows, increasing the He pressure from a few psi to 65 pounds (or recommended pressure for instrument used), igniting the flame, turning on the recorder, turning on the flame voltage, and setting the sensitivity switch to 1×10^{-10} amps/mv from "balance". The column is heated from 75° to 275° for 15-20 min., cooled to 75°, and the methane retention time (MRT) is measured. If necessary (rarely) the carrier gas flow is adjusted to maintain the MRT at 44 ±0.5 sec by adjusting the split ratio.

Only if the pen returns accurately to the same signal at 75° C without bucking can baselines be applied to samples. The instrument "balance" is set and checked according to the manufacturer's recommendations. Pen deflection without bucking should be very small (50 microvolts on the 1 mv scale). Failure of accurate return may be caused by dirty detector

excessive column bleed, or too short cooldown (5 - 10 mins. minimum). The small reproducible signal at 75° is "bucked out".

Samples, baselines, and standards should be analyzed in an order that facilitates making comparisons by placing similar samples together and evenly spacing baselines, standard #2 fuel oil, or any other standards. For very close comparison, samples are analyzed with sample size being adjusted to yield comparable sized chromatograms, determined by a sample-size/peak ratio curve as in Figure 5. A maximum signal intensity of 0.5 - 1.0 mv is satisfactory for routine work. If the sample size recommendations regularly yield too large or small injections, the split gas flow should be increased; keep the MRT at 44".

Each analysis is begun by:

Starting recorder chart drive,

Injecting solution with a 5 or 10 ul syringe as pen approaches a chart division. Mark chart by momentarily moving electrometer range switch while needle is still in injector. Injection techniques vary; be consistent. Withdraw the syringe plunger by ½ the needle volume after loading the syringe so that an air plug fills the front half of the needle; *sample fractionation occurs if the full needle is brought near the hot injection port.* Avoid injecting excessive volumes of air.

Start the program when the solvent peak begins to elute. After the program is completed, maintain 275° until the detector signal returns to the level of the previously determined baseline, or for 35 mins., whichever is sooner. Cool the column back to 75°, reset the programmer, cut the chromatogram off, and hang the weight (if one is used) on the recorder chart.

The recommended standby condition is: air and hydrogen off, helium pressure reduced to a few pounds (CAUTION: gauge readings stabilize slowly. Do not turn off completely), the flame voltage off, electrometer at "balance". The recorder electronics may be left on or off. The oven is set at 75° C.

5. Maintenance Procedures and Standards

The following procedures are performed when needed.

Replace the septum every 25 injections or when needed, (leaks, erratic MRT, bleed peaks). Precondition septa to minimize bleed by storing them in the column oven in a beaker.

Clean detector as described if symptoms of dirty detector appear, especially "spiking" or failure to return to low signal level after cooling column back to 75° C.

Replace injector liner and clean dirty one in CS_2 every 3-4 injections or more frequently if high-residue oils (crudes, asphalts, heavy fuels) are being analyzed.

Clean the injector by the procedure given in Appendix 4 at intervals of 100 injections or whenever RSD's of ratios involving peaks of different boiling points increase. The increased variability is a sign that tars or glass fragments are in the injector tubing and require flushing out.

As column bleed stabilizes, occasional (every 60 or so injections) series of replicate standard #2 oils should be run 4 - 6 times to establish short-term RSD values and to compare with earlier performance. The stan-

dard oil should be run at least every 20 injections.

Determine a baseline curve (CS_2 injection, then program) every 25 injections or when isothermal signal at 275° changes 5% or more.

6. Chromatogram Measurement and Data Tabulation

Comparison of details of chromatograms requires direct visual comparison of traces obtained under comparable conditions. However, measuring and tabulating certain signal intensity ratios abstracts most of the useful information in a form suitable for simultaneous comparison of many samples and for statistical purposes. All useful chromatograms should be tabulated.

First, a baseline curve is superimposed on the sample chromatogram of interest, then the vertical distances at selected points between baseline and chromatogram signal are measured and recorded, and finally signal intensity ratios are calculated and tabulated (Fig. 1,Table 7). The baseline curve should be obtained from the same instrument/column combination. If the column and septa are well conditioned, and if the daily bake-out procedure is followed, the baseline should be a smooth curve devoid of peaks. The absolute signal level (in mv) at the end of the temperature program should be constant. Within a few percent, the same signal level should be asymptotically approached at the end of a sample chromatogram as the sample elutes isothermally. These criteria are generally satisfied for a period of 25+ injections by a single curve; however, a new baseline should be determined after each septum change to guard against effects from materials vap-

orizing out of the septum. Ratios should not be formed at locations at which septum bleed peaks appear without extremely frequent baseline checks (cold columns collect septum bleed); in our experience, septum bleed peaks have occurred at considerably higher elution temperatures than the signals we ratio.

The baseline curve is traced accurately onto the sample chromatogram, using the rising edge of the solvent peak as a fiducial mark and further aligning the chromatograms by the 0 and 100% scale lines. We repeat that it is extremely important that programs be started uniformly, that programmers track correctly, and that recorders feed paper without slippage. We have found that mechanical slippage within programmers may occur; the programmer should be timed frequently during at least one full revolution of the indicating dial to see that it advances at a reproducible rate.

Accurate vertical distances along the millivolt axis are measured with a glass and ruler calibrated in hundredths of an inch, with care to avoid parallax error. Different observers rarely disagree by more than ±0.01". The most useful ratios require measuring the heights of the $n\text{-}C_{17}$, pristane, $n\text{-}C_{18}$, and phytane peak above the traced baseline. These peaks elute in the order mentioned in our system, and can often easily be recognized by eye in a chromatogram (Figure 1 and Appendix 2). They may also be located to within $\pm 1^{\circ}$ C by measuring the distance from the solvent peak and comparing with the peaks of standard fuel oil or a standard sample such as spiked Bachaquero crude oil (Chromatogram 15). These peaks are inconspicuous in such rare oils as Bachaquero crude; the sample may

be re-analyzed with a small added spike of the standards (chromatogram 15).

The analyst should recall that the designations of these peaks are names and not strict chemical identifications, and also that signal intensity measurements, rather than area measurements, are made: it is erroneous to view the ratios as being accurately related to the amounts of the named substances present in the sample. Also, though it is convenient to form four of the possible ratios, only any three of the four are independent parameters.

It is also helpful to tabulate a measure of the extent to which the signal consists of a very complex "background" of unresolved substances. A convenient but arbitrary measure is formed by drawing a tangent line to the valleys immediately preceding the n-C_{17} and n-C_{18} peaks, and measuring the distance from the baseline to this line along the vertical which passes through the n-C_{17} peak. We recommend replacing this ratio, or supplementing it, with a ratio based on the vertical which passes through the phytane peak, the tangent line being extended slightly to underly this peak. This latter ratio is less subject to alteration by evaporative weathering or by bacterial action on the oil than is the n-C_{17} background ratio.

The ratios should be formed to four significant figures, but rounded to three unless they are to be averaged or utilized in statistical estimates. Further ratios may be found useful in particular applications. A useful approximation is: if two chromatograms do not differ with respect to a feature by visual inspection, they will rarely differ significantly when

measured and tabulated. Often, samples differ very widely in their overall boiling point distribution even though they are similar in some region. It often suffices to sketch the overall envelope shape in the "remarks" column of a tabulation sheet, rather than create many new ratios to measure this.

Within the sample sizes recommended, the sample size has only a small effect on ratios. Figure 5 summarizes the results of a study of sample size vs. ratio values performed on column 219 using the standard #2 fuel oil. For work with very similar samples, or where multiple runs are utilized, sample size-ratio regression lines could be utilized to increase accuracy, particularly for the peak/background type ratios which are most sensitive. More information could also be gained by standardizing sample preparation and introduction so that absolute signal sizes could be accurately compared; however, this would require considerable effort with little probable gain.

When large numbers of samples are analyzed with little foreknowledge of their type, many traces may have poor signal intensities. One can estimate sample sizes on a large number of samples by running an old column isothermally at high temperature and injecting the samples (injector 50° C hotter than oven) at fairly short time intervals in another instrument. The column used will deteriorate rapidly and the instrument injector/splitter will require cleaning.

7. Artificial Weathering

Evaporative weathering can be simulated in the laboratory to determine whether this agent could have caused observed differences between a sample

and suspected source. The procedure given below simulates almost exactly changes in source oils EPA exposed to wind; for example, see chromatograms 7, 8, 9. The changes after 30 days were a 15% drop in the $n\text{-}C_{17}/n\text{-}C_{18}$ and the pristane/phytane ratios and alterations of fine structure in the portions of the chromatogram below the elution of n C_{18}.

The oil sample to be weathered is placed in a flask and connected to a rotary evaporator. Vacuum is applied slowly until a full mechanical pump vacuum can be applied without excessive foaming or bubbling, and evacuation is continued while the temperature of a water bath surrounding the flask is raised slowly. Small samples are withdrawn periodically for GC analysis. Several hours pumping at below 1 mm Hg in a bath at 60° C or warmer may be required to yield much evaporation in the C_{17} region. A sample which resembles the environmental sample in degree of evaporation (as judged by the comparison of shape of the low-boiling-range signal or a ratio of background at C_{15} to any signal parameter at C_{17} or C_{18}) is tabulated to determine the shift in analytical ratios caused by evaporative weathering. This method should be applied with caution if ratio changes greater than 20% are required; such samples are heavily weathered.

INTERPRETATION OF DATA

The Introduction presented the possibilities and limitations of correlating oil by chemical methods. This section presents more detailed guidelines for correlating weathered oils with suspected sources by gas chromatography.

Straightforward situations frequently arise: a single clear correlation exists, no source matches the spill, or several indistinguishable possible source oils exist. The following guidelines are designed to minimize false correlations--the worst possible error--when this possibility arises. Some situations leading to false correlations occur if: an environmental sample which is an oil or oil-control chemical mixture is mistaken for a single oil and the ratios correlate with a possible source; unusually intense bacterial alteration affects ratios, especially those involving n-hydrocarbons; the actual source is missing but the sample is correlated with a very similar suspected source.

The major factors the analyst must always consider are the completeness and validity of the sample collections, the effects of weathering on the environmental sample, and the ability of the analytical technique to distinguish different oils. Table II lists several types of problems which may be encountered.

The distinguishing power of the technique can be estimated and is therefore the least serious problem. Estimates of the RSD's of various ratios can be made by replicate analyses; the RSD values of ratio means for the standard oil apply approximately to the sample. By analyzing the primary standard samples (see page 14) or secondary working stan-

TABLE 11

Problems in Oil Spill Correlation

UNREPRESENTATIVE SAMPLES

- Missing sources
- Unrepresentative source samples
- Unrepresentative spill samples
    - Admixed spill control chemicals
    - Mixed spills
    - Mixed with indigenous hydrocarbons

UNRESOLVABLE PAIRS

- Multiple samples of the same oil
- Similar oils, especially crudes
- Inadequate resolution

ENVIRONMENTAL ALTERATION

- Unusual bacterial alteration
- High levels of indigenous hydrocarbons
- Mixtures
- Severe evaporation or old samples
- Unknown processes

dards more suitable to the investigation at hand, the analyst checks the instrumental and measurement steps, and most major sample preparation variables. The information gained by these tests should be utilized--qualitatively by the exercise of judgement or quantitatively by applying simple statistical tests--to judge whether observed differences in sample ratios are significant, or whether they might easily have arisen by chance. We have utilized Student's "t" test to compare data.

The remaining important factors--sample collection validity and weathering effects--are not subject to such rigorous control. We recommend that routine correlation of oils with sources be limited to samples young and oil-rich enough that these possibilities are minimized. Oils, oil slicks, "tarballs", oil-rich water, and oil collected as discrete coatings on sand, sediments and organisms, etc. will generally have a negligible indigenous substrate hydrocarbon content. These larger masses of oil are also unlikely to be strongly affected by bacteria or to become thoroughly mixed with other oils in short time periods. Oils estimated to be less than a month old are most promising for correlation. The source collection for older oils is likely to be incomplete, and weathering effects will be more pronounced. EPA-sponsored work proceeding at Portland, Maine, is expected to yield better estimates of the period during which correlation has minimal chance of error. A large percentage of the situations of interest in water-quality management are weeks-old oil-rich samples suitable for routine work.

In contrast, oil extracted from dilute water samples, sediments and organisms is more likely to be significantly weathered and accompanied by an admixture of indigenous hydrocarbons. These samples require preliminary

separations and parallel background determinations on control samples; extensive and intensive sampling programs may be required to estimate the extent of weathering, and the probable source collection is difficult to complete if much time is elapsed. We recommend that only laboratories specializing in oil analysis consider such samples for routine correlation. The analyst e[illegible]ed in this work must be capable of applying the general criteria se[illegible]orth in the introduction to the situation at hand and have a sound kno[illegible]ledge of oil chemistry and the chemistry of environmental lipids.

However, it is possible to establish routinely, the presence of oil in sediments and organisms even when correlation with specific sources on chemical evidence alone is unwarranted; after a major spill, this presence may be important in establishing the fate and effects of oil not requiring precise correlation.

For routine analysis of samples, a set of chromatograms of the environmental sample and all available possible sources is required. This data set should be of the maximum feasible comparability, especially if similar-appearing samples are involved. For close comparisons, a standard sample of #2 fuel oil and a second subsample of the spill (not re-analysis of the same solution) should be run so that the entire group is bracketed by these analyses. If alcohol-type spill control chemicals have been used, the samples must all be column chromatographed as described for organisms and sediments; hydrocarbon-base spill control chemicals cannot be removed. In favorable cases, mixed samples may be treated as a linear combination to derive ratios for the spilled oil.

If the two analyses of the subsamples of spilled oil are similar within the expected analytical error, correlation can be begun. Otherwise, either the performance of the method or the representativeness and homogeneity of the sample are deficient; check method performance and remedy defects. Analysis of additional subsamples or sample recollection may be necessary.

The mean values of the analytical ratios for the two spill sample analyses are calculated, and the onset of appreciable signal in the spill sample chromatograms is used to judge evaporation effects. If the onset of signal in the environmental sample has actually been determined by the composition of the original oil, an exact correspondence of spill and source chromatograms will exist; the source oil will then be a material free of low-boiling components. If the onset of spill sample signal is below n-C_{14}, none of the tabulated ratios should be affected by evaporation; onset of signal at or below n-C_{15} implies alteration of the ratios which can be simulated by artificial weathering; onset of signal beyond n-C_{16} implies relatively strong alteration of ratios other than 17/pris, 18/phy, and ratios to background signals at higher boiling points. Such evaporated samples are probably more than several weeks old.

The environmental sample is compared with each suspected source in turn. The spill and its source should match after the allowances suggested above are taken into account. It is particularly important to compare <u>all</u> sources, and not to identify the spill sample with the first matching source encountered until all others are ruled out. The "possible source" list is narrowed by rejecting samples with any markedly different (5-6x RSD) ratios, different boiling-point distributions (envelope shape) or

patterns of smaller peaks. These rejections simply narrow the field; though in simple cases it narrows the possible sources to one. Generally, the rejections drastically narrow the possible source oils. If further comparisons are necessary, repeat analyses of the more similar oils and determine whether significant differences between source and sample exist according to some statistical measure, such as Student's "t" test. At this stage, it may be desirable to artificially weather by evaporation the candidate sources for closer comparison of the 17/18 and pristane/phytane ratios.

We define a *unique* correlation as one for which all but one possible source is ruled out, and the one source does not differ significantly (after artificial weathering if necessary) from the environmental sample. Replicate analyses for better comparison may be performed.

For samples which seem to correlate except for ratios involving the n-hydrocarbons, the possibility of unusually rapid microbial catabolism must be considered. This will lower the n-hydrocarbon peaks by a nearly uniform amount over the entire chromatogram, and thus lower the corresponding ratios. Microbiological attack on other oil components is much slower, and correlations established with at least one non-normal ratio are correspondingly more secure.

Each correlation should be reviewed on the basis of the assembled evidence. The possibility that any one of the possible sources was unusually rapidly attacked by microorganisms, lowering the corresponding ratios, should be considered. The other features (envelope shape, small peak patterns) of the chromatograms should be checked carefully to confirm

that no information contradicts the assignment made.

We have inserted cautionary comments repeatedly about n-hydrocarbon oxidation by bacteria. This is primarily because laboratory experiments have shown that rapid (days) alteration of oils is possible. On the other hand, the weight of evidence suggests that the ideal conditions required are rarely achieved in the natural environment. Laboratory experiments often involve conditions rare in nature: high nutrient concentrations, mixed cultures, aeration, the availability of additional energy sources, and strong agitation to disperse the oil. Oil films on hay or decaying seaweed may naturally alter relatively rapidly. However, the alteration of larger masses (centimeter dimensions) of oil in a few weeks time has yet to be observed.

Finally, the analyst should check his conclusion against adjunct information about the spill, its possible sources, and the conditions which the sample withstood. With experience, he can streamline procedures to minimize duplicate analyses and compare samples with known locally abundant materials; the ratio stability and comparability afforded by the use of long-lived SCOT columns and standards permits this approach. In the case of important or difficult situations, or in research applications, the analyst should also use additional techniques, as are discussed in the next section.

ANCILLARY TECHNIQUES

Although a GC method will often yield a conclusive result in oil correlation problems, cases requiring confirmatory evidence or distinguishing an oil pair not distinguishable by GC will arise. The information gained from gas chromatography can assist in solving these problems also, either by further use of the capabilities of GC, or by applying information from the gas chromatogram to select suitable further procedures.

Higher resolution use of gas chromatography is one possible approach. The use of a second detector, such as an S-sensitive flame photometric or electron-capture detector, would yield more information on specific parameters without additional time or effort. The SCOT OV-101 column is ideally suited (good resolution, low bleed, low gas flow) for GC-MS instrumentation; mass spectra may have distinguishing features not clear in the FID trace alone. Low voltage MS or GC-MS is undoubtedly the best way to demonstrate unequivocally the presence of oil-derived aromatic hydrocarbons in organisms. The capability of gas chromatography may also be extended by applying preliminary separations before analysis: separation of aromatics (several groups) and saturates by adsorption chromatography or separation by molecular shape (clathrates, molecular sieves) before gas chromatography are proven techniques (Eglinton & Murphy, 1969). Preparative gas chromatography on packed columns may also be used to obtain fractions for analysis by other methods.

A second way of utilizing GC in conjunction with other techniques is to take advantage of the information gained by GC. The number of samples to be analyzed by expensive or complex techniques can be drastically reduced by using GC analysis as a very high performance screening procedure. Detailed examination of samples by high-resolution or low-voltage mass spectroscopy, by neutron activation analysis, or by various isotopic measurements may only

be economically feasible with the use of screening to reduce sample load. The neutron activation method (Lukens, 1971) may be particularly promising and complementary to GC; the trace elements analyzed may enable one to distinguish identical oils shipped in different vessels, pipelines, etc., by virtue of the pickup of a trace element "fingerprint".

The boiling-point distribution indicated by GC indicates some information useful in ruling out nonproductive techniques. Fuel oils will often tend towards legal S content limits; S analysis is not promising in distinguishing them. Ni and V contents of lower-boiling distilled products will be very low. Estimates of the extent of evaporative weathering by GC may help in evaluating data from many other methods, such as infrared, ultraviolet, and fluorescence measurements. The similarity of the boiling point distribution curve to that of some known product type (see Appendix 2) is useful in estimating the possible sources in a given area.

The work on petroleum-derived hydrocarbons in marine lipids by IDOE has also shown that GC analysis is the best procedure for determining the possible presence of petroleum hydrocarbons in organisms. The oil-derived contribution is detected as the complex, unresolved envelope. Such samples are best examined further by techniques such as GC-MS and low-voltage mass spectroscopy.

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John Farrington is the author of Appendix 3.

APPENDIX I

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Articles of special interest and background materials are marked by an asterisk in the left-hand margin.

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APPENDIX 2

SAMPLE CHROMATOGRAMS

Chromatogram Number	Column Number	Injection Number	Description
Column Baselines			
1	218	74	
2	218	132	
3	218	148	
Standards #2 Fuel Oil			
4	216	124	
5	218	149	
6	219	181	
Short Term Weathering Study			
7	219	57	So. Louisiana Crude
8	219	61	So. Louisiana Crude--Weathered 24 h.
9	219	60	So. Louisiana Crude--Weathered 1 mo.
Spill Control Chemicals			
10	218	77	Magnus Dispersant
11	218	123	W. G. Smith Surface Collecting Agent
12	218	122	Shell Herder
13	219	62	#6 Fuel Oil with Shell Herder
C-17, C-18, Pristane and Phytane			
14	219	111	Mixture only
15	219	112	Mixture with Bachaquero Crude
Carrier Gas Effects Study (#2 FO, STD)			
16	219	207	With Helium
17	219	209	With Nitrogen
18	219	210	With Nitrogen and Reduced Flow Rate
Crudes			
19	219	57	So. Louisiana
20	219	158	Lago medio
21	219	161	Lago mar
22	219	145	Agha Jari
23	219	174	Lago treco
24	219	163	Ceuta
25	219	160	T. J. Light

Chrom. No.	Col. No.	Inj. No.	Description
26	219	144	Nigerian
27	219	155	Nigerian / F. O.
28	219	164	Guanipa
29	219	146	Orito
30	219	168	Zarkum
31	219	170	Qatar
32	219	173	Kuwait
33	216	156	T.J. 102
34	216	158	Murban
35	219	77	La Rosa
36	219	94	Lagunillas (Lago)
37	218	62	Arabian Light
38	218	22	Chevron Ventura Crude
39	219	197	Miss. - Lime
40	219	199	Penn. - Strawn, Stevens Co. Reg.
41	219	200	Penn. - Strawn, Eastland Co. Reg.
42	219	35	Bachaquero
43	219	202	Ordovician-Ellenburger
Petroleum Products			
44	218	152	Gasoline
45	218	157	Kerosene
46	218	149	#2 Fuel Oil
47	218	158	Marine Diesel
48	219	178	#4 Fuel Oil
49	219	183	#6 Fuel Oil
50	218	159	Asphalt
51	218	24	Crankcase Oil (Used)
"Dexsil 300" Column			
52	220	3	#2 Fuel Oil Standard

DATA FOR OIL AND OIL PRODUCTS EXEMPLIFIED IN APPENDIX 2

Chromatogram #	Column No.	Signal Intensity Ratios					Comment
		Pris/ Phyt.	$n\text{-}C_{17}$/ Pris.	$n\text{-}C_{18}$/ Phy.	$n\text{-}C_{17}$/ $n\text{-}C_{18}$	$n\text{-}C_{17}$/ B.G.	
1	218	-	-	-	-	-	d
2	218	-	-	-	-	-	d
3	218	-	-	-	-	-	d
4	216	1.83	1.29	1.65	1.44	3.42	b
5	218	1.81	1.41	1.90	1.34	4.36	b
6	219	1.88	1.41	1.90	1.40	4.34	b
7	219	1.61	1.34	1.68	1.28	4.97	c
8	219	1.67	1.31	1.65	1.31	5.10	a
9	219	1.61	1.29	1.67	1.25	5.19	a
10	218	1.35	2.02	2.04	1.33	5.25	a
11	218	-	-	-	-	-	d
12	218	-	-	-	-	-	d
13	219	1.37	1.54	2.07	1.02	3.50	a
14	219	-	-	-	-	-	d
15	219	-	-	-	-	-	d
16	219	1.83	1.41	1.89	1.37	4.01	a
17	219	-	-	-	-	-	e
18	219	-	-	-	-	-	e
19	219	1.61	1.34	1.68	1.28	4.97	c
20	219	0.87	2.06	1.54	1.16	5.31	c
21	219	0.86	1.96	1.52	1.12	4.90	c
22	219	0.96	1.94	1.57	1.19	5.77	c
23	219	1.01	1.88	1.68	1.12	4.42	c
24	219	0.98	1.78	1.58	1.12	4.08	c
25	219	0.95	1.86	1.57	1.12	4.44	a
26	219	2.03	1.28	2.02	1.28	5.79	c
27	219	2.00	0.85	1.40	1.22	3.26	a
28	219	1.98	1.07	1.79	1.17	3.84	c
29	219	1.14	1.30	1.19	1.24	5.14	a
30	219	0.85	3.67	2.85	1.10	6.68	a
31	219	0.88	3.41	2.60	1.15	6.38	a
32	219	0.82	2.95	2.08	1.15	5.87	c
33	216	~1	~1	~1	~1	~1	a

Data (Continued)

		Pr/Ph	17/Pr	18/Ph	17/18	17/BG	
34	216	0.94	2.58	2.23	1.09	-	c
35	219	0.96	1.34	1.18	1.09	2.73	c
36	219	1.00	1.01	1.08	0.94	1.48	c
37	218	0.94	1.93	1.58	1.15	5.90	c
38	218	1.29	0.84	0.98	1.10	5.23	a
39	219	1.29	1.38	1.51	1.18	3.98	a
40	219	1.36	1.21	1.35	1.22	4.67	a
41	219	1.36	1.04	1.16	1.18	3.81	a
42	219	0.85	0.96	0.99	0.84	1.02	a
43	219	1.20	1.55	1.61	1.15	5.33	a
44	218	-	-	-	-	-	d
45	218	-	-	-	-	-	d
46	218	1.81	1.41	1.90	1.34	4.36	b
47	218	0.87	1.37	1.29	0.92	2.86	a
48	219	2.06	1.39	2.04	1.41	7.41	a
49	219	1.41	1.40	2.07	0.95	5.83	a
50	218	-	-	-	-	-	d
51	218	-	-	-	-	-	d
52	220	-	-	-	-	-	e

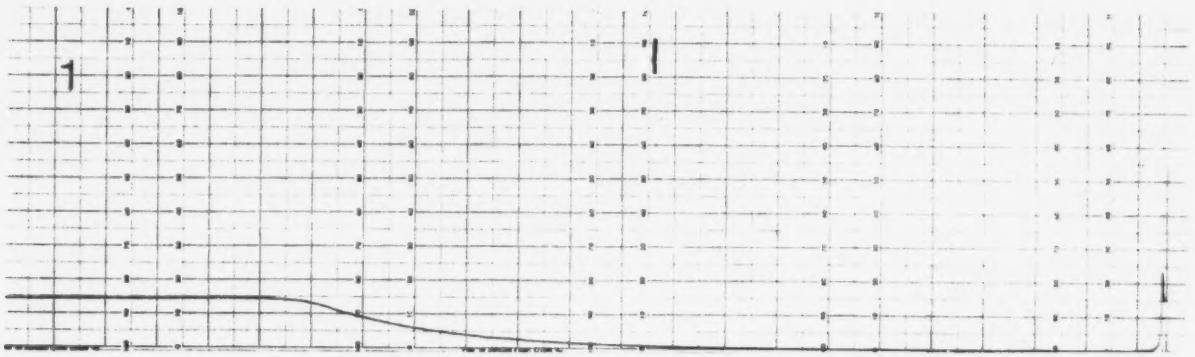
a Values reported are for single determination.

b Values reported are averages of 11 - 13 determinations.

c Values reported are averages of 2 - 5 determinations.

d Samples were not oils or oil products containing any of the desired components.

e Poor resolution.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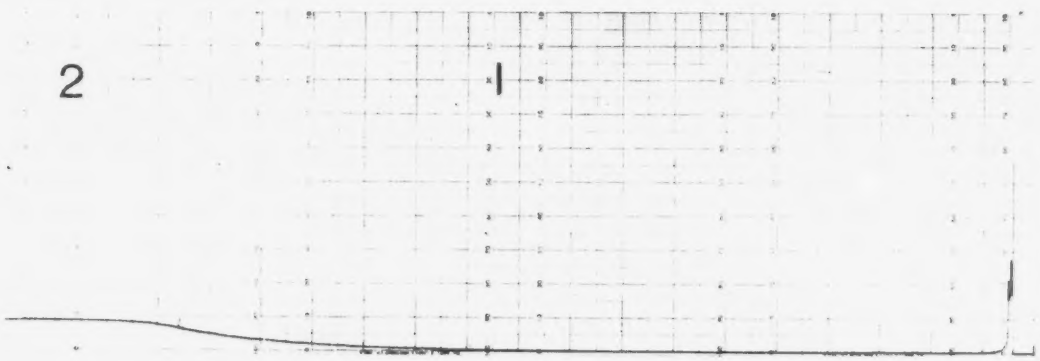
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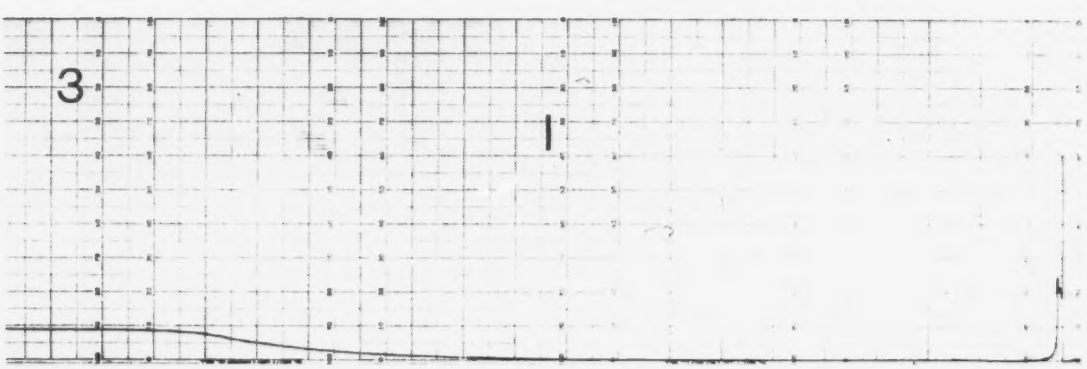
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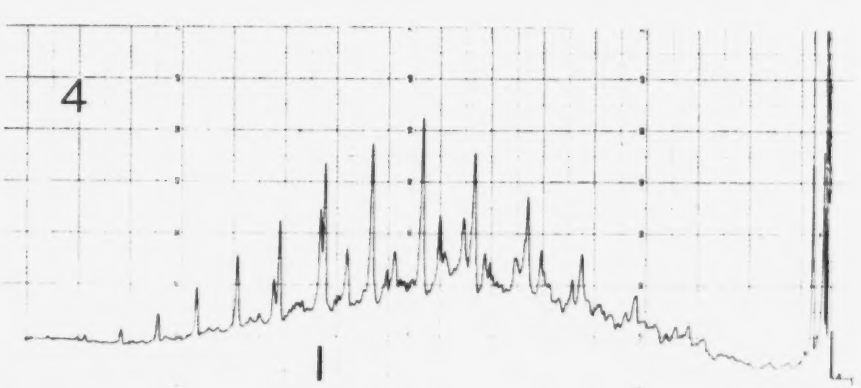
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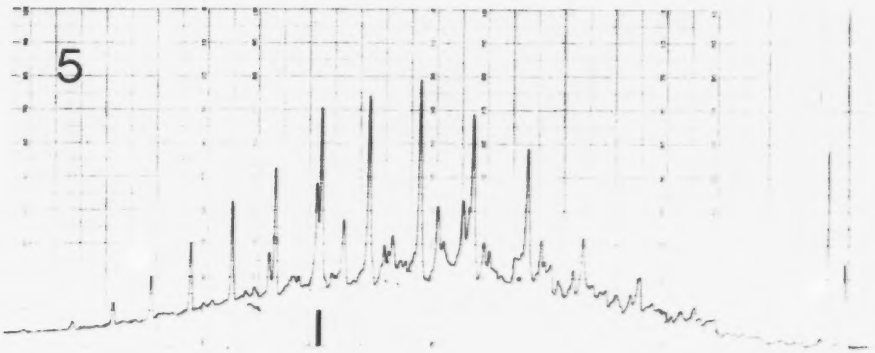
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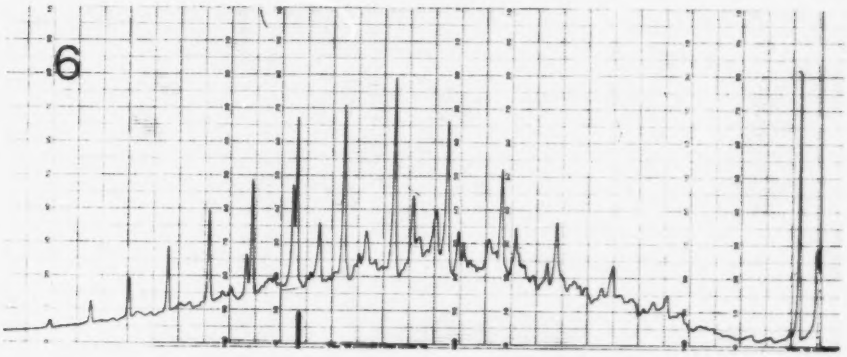
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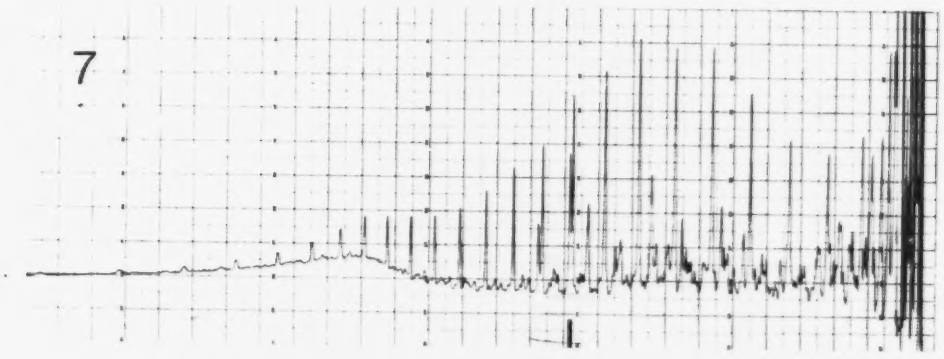
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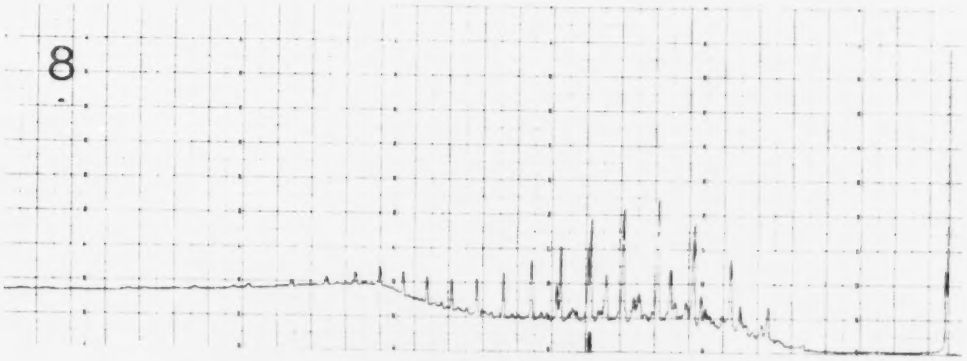
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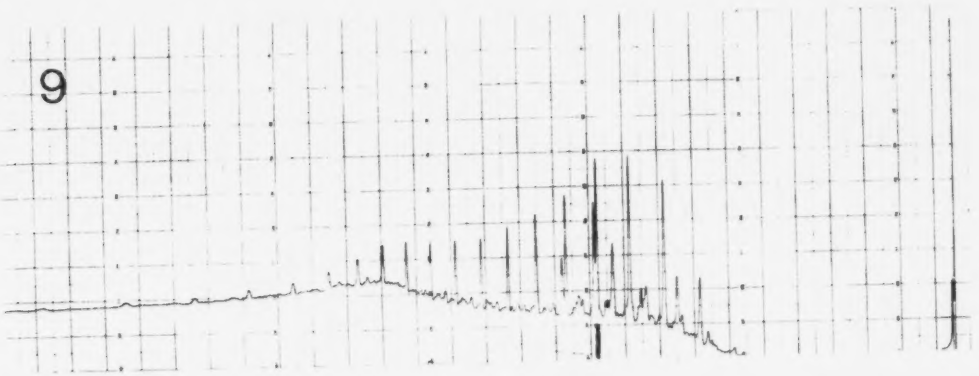
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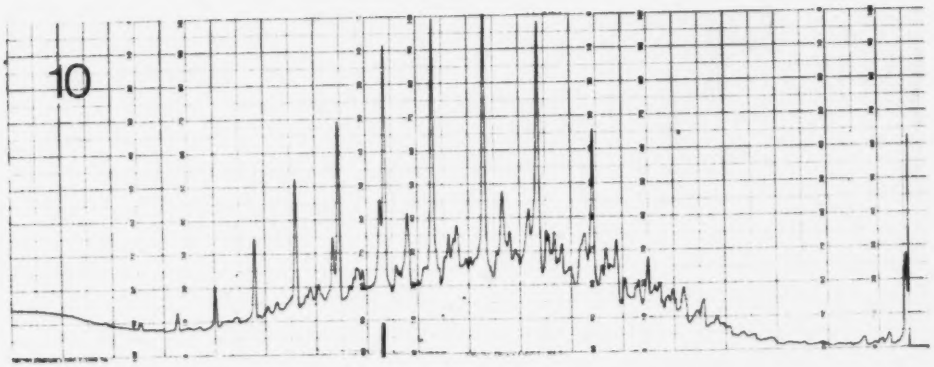
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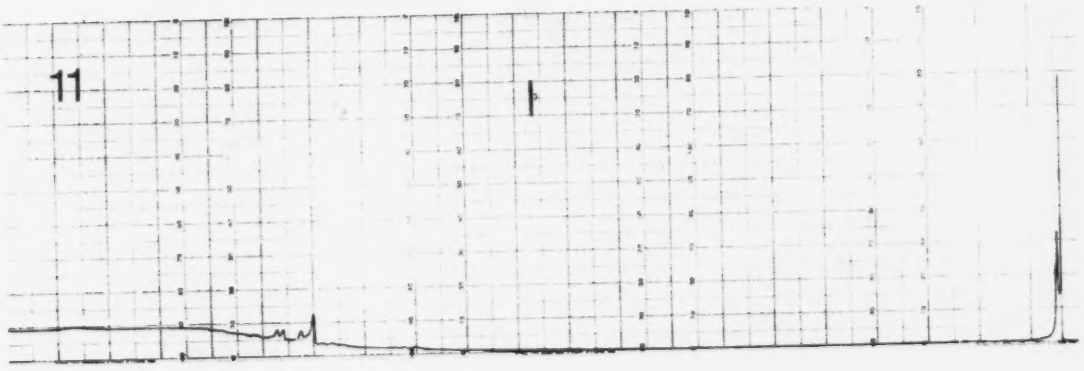
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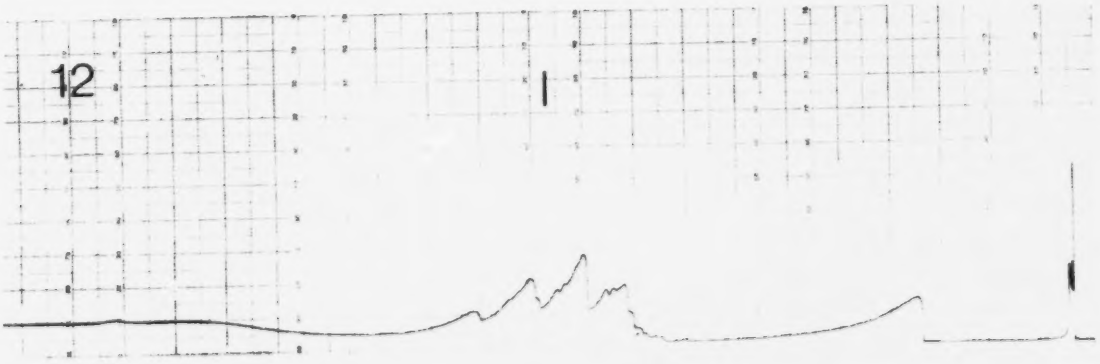
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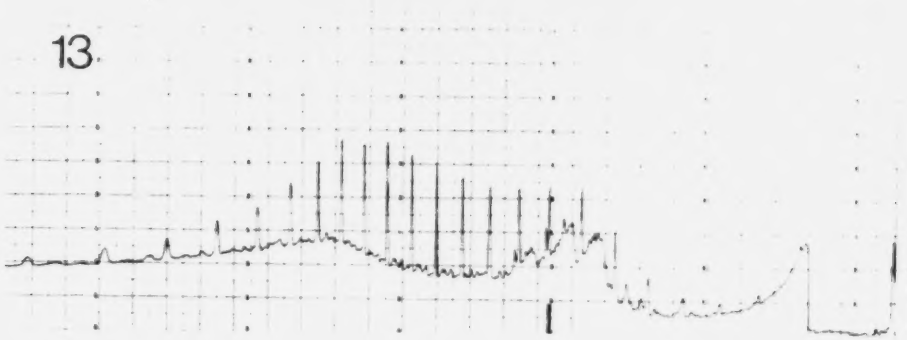

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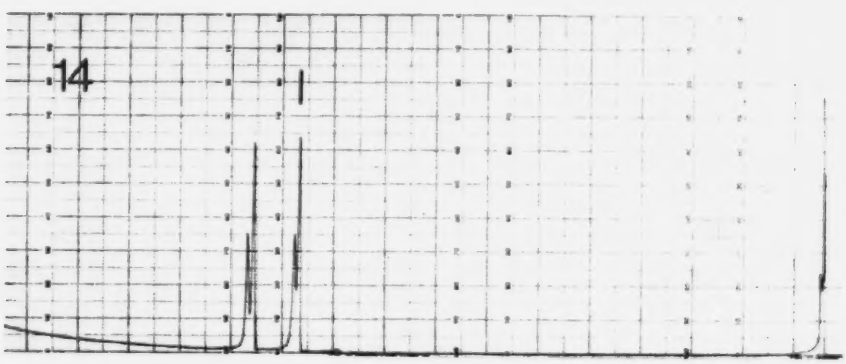

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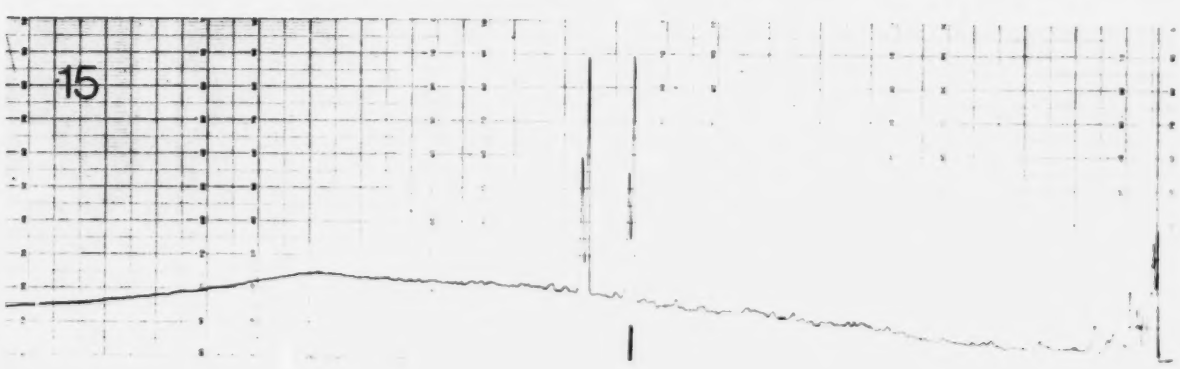

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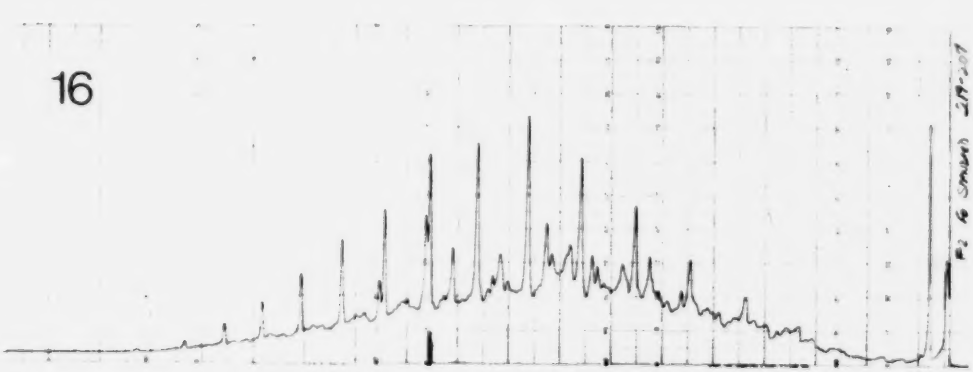

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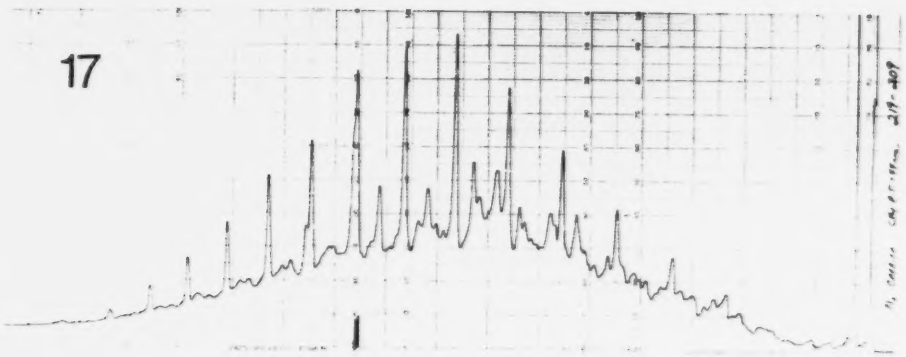

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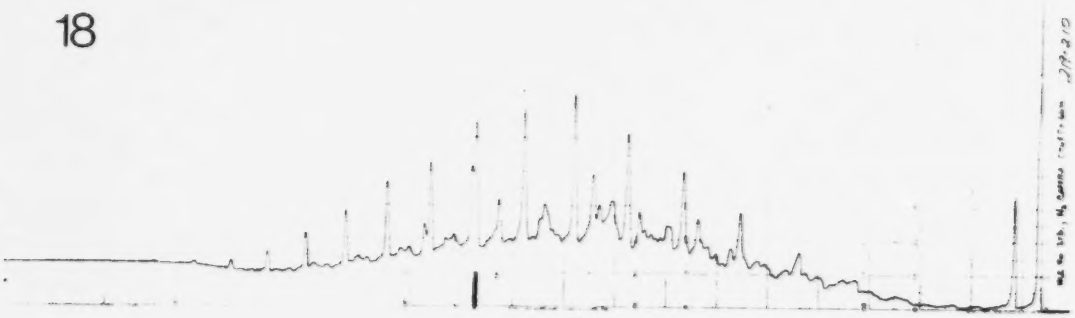

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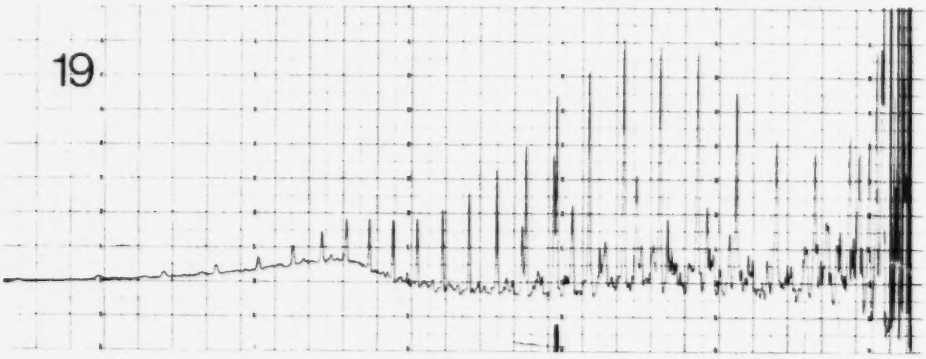

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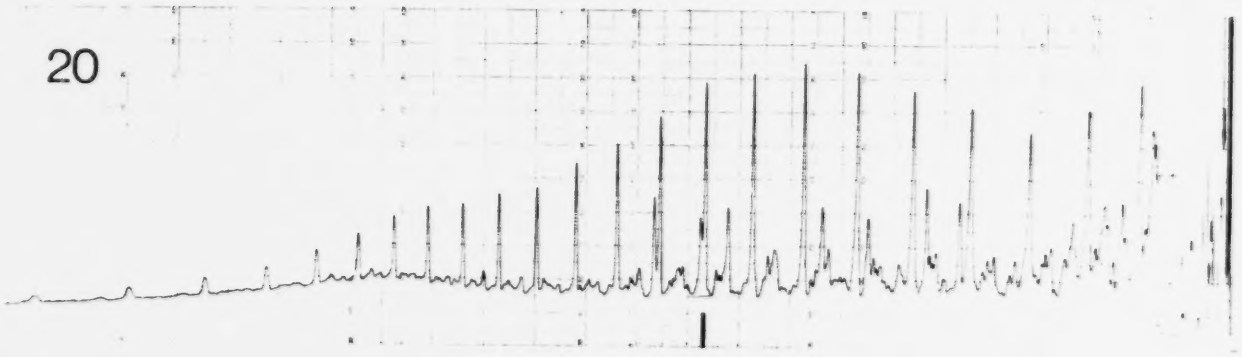

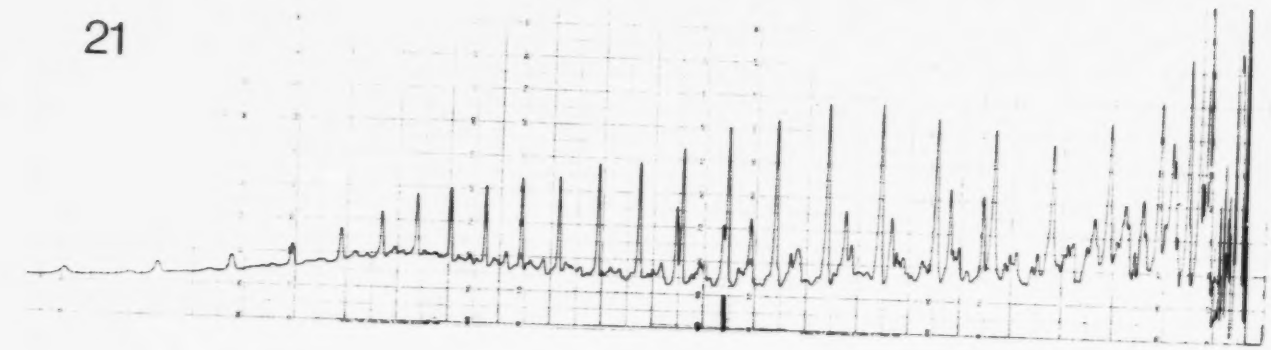
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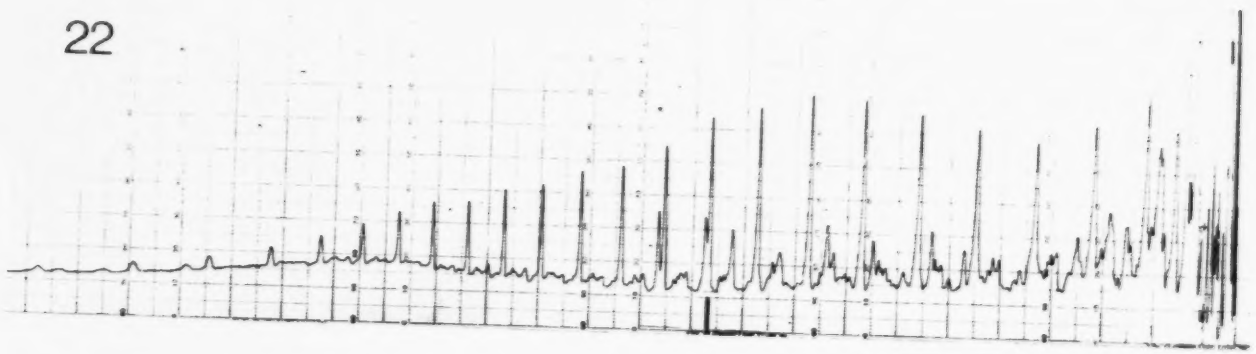
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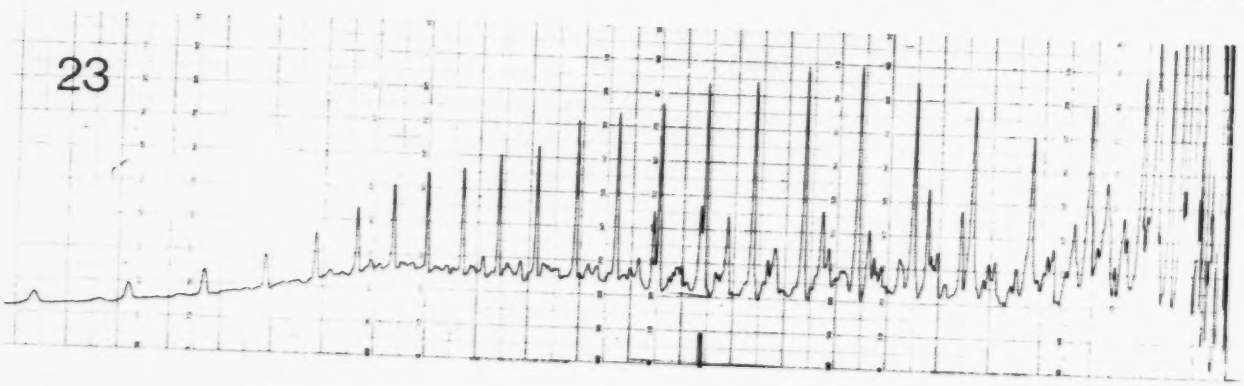
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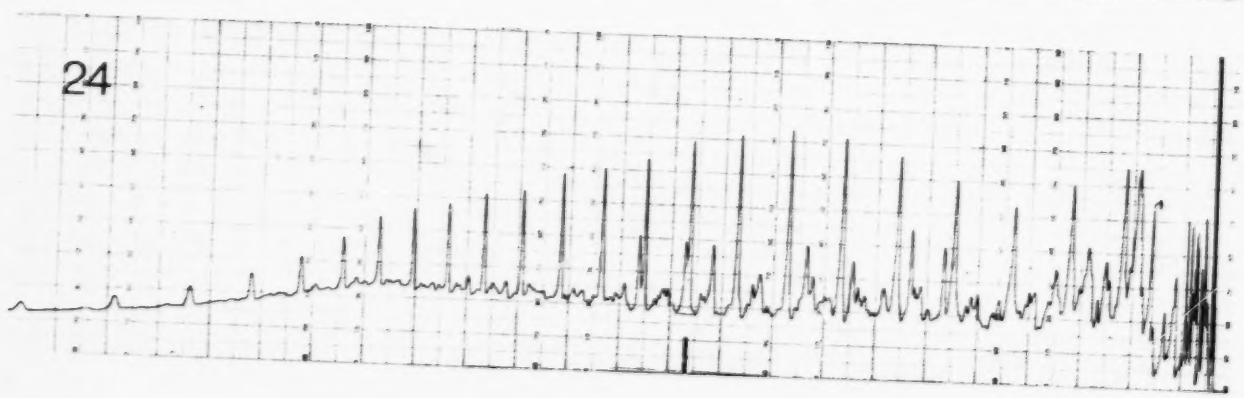
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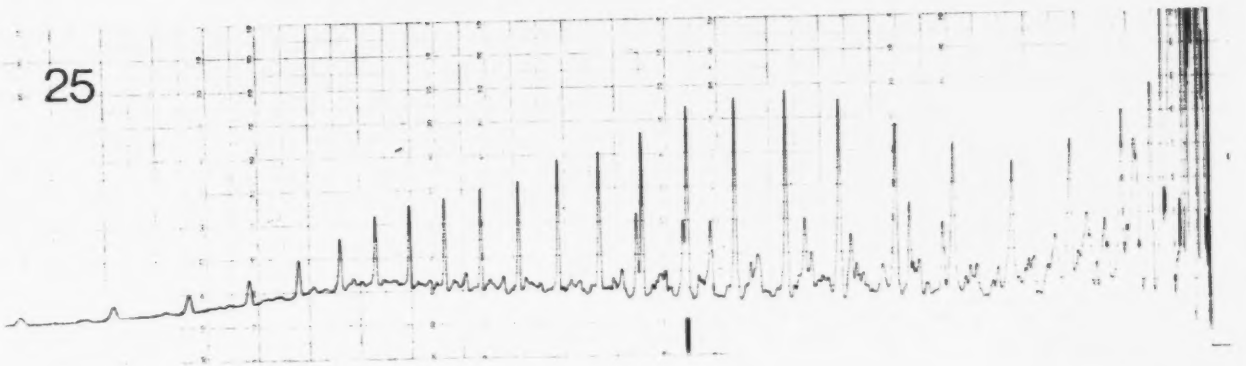
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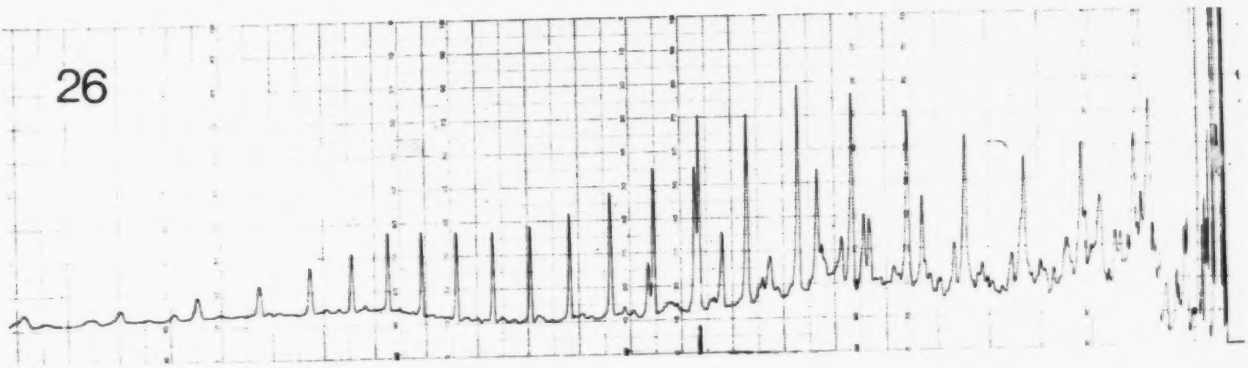
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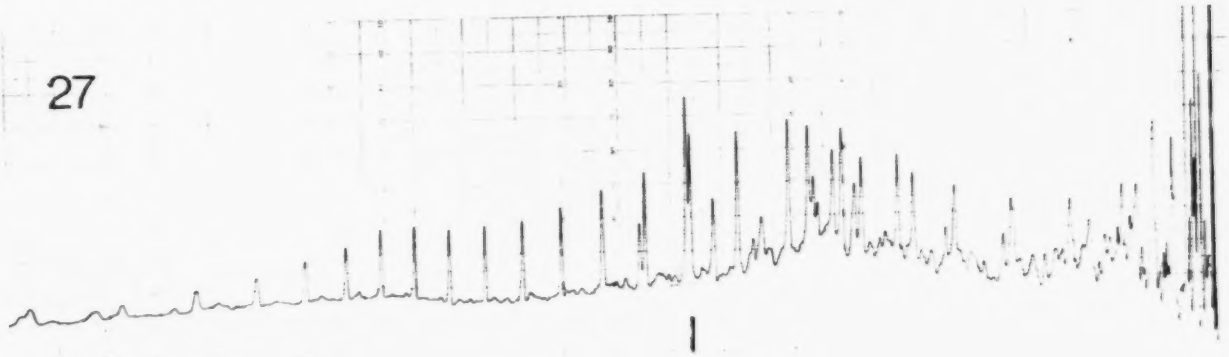
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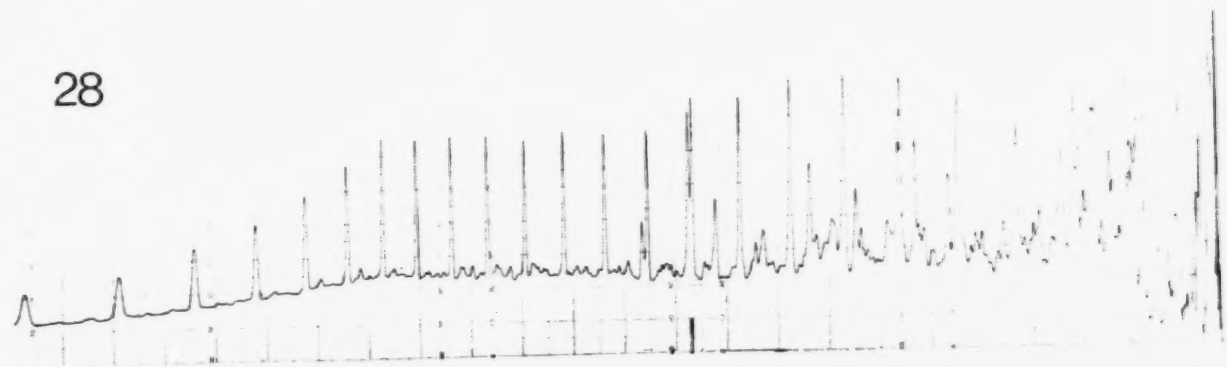
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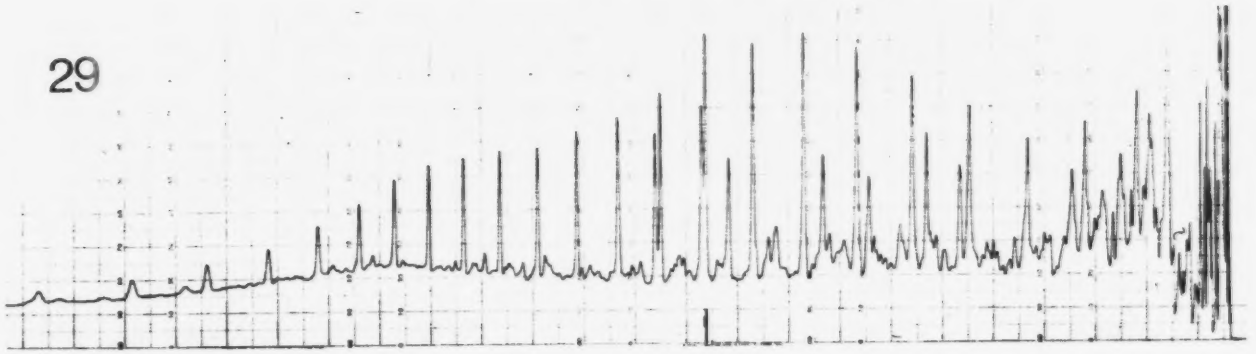
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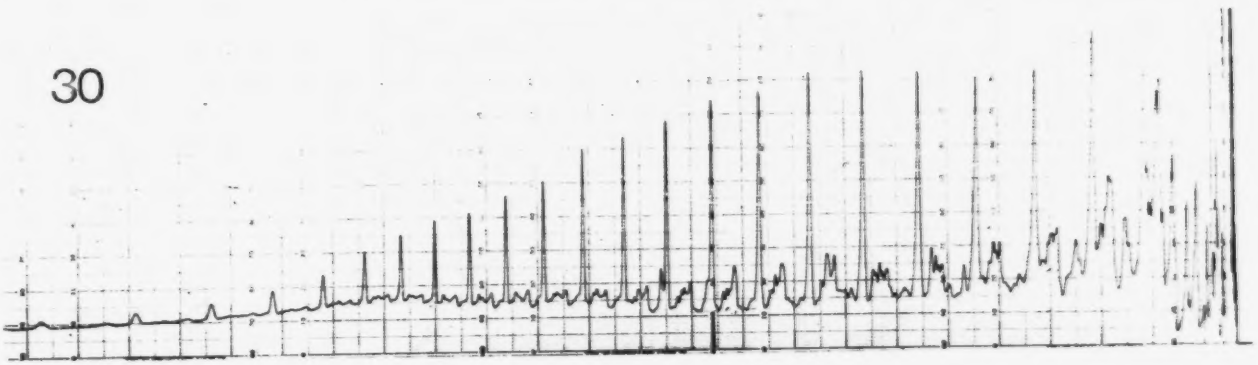
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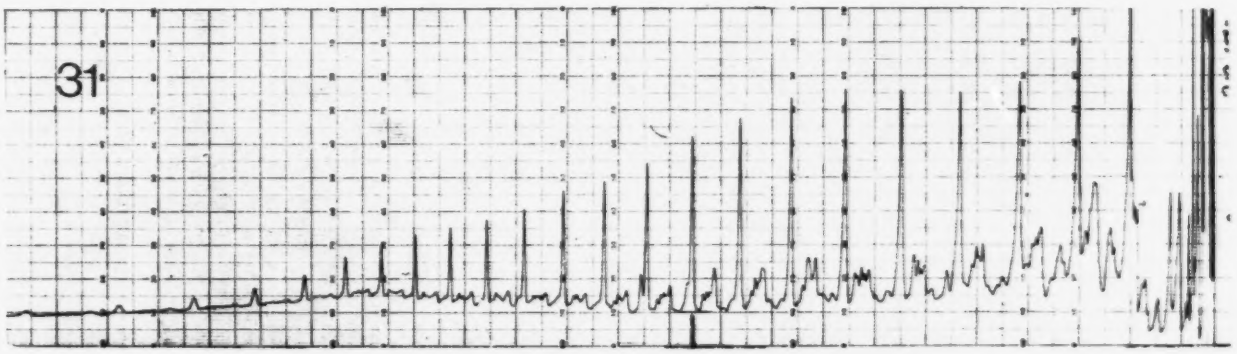
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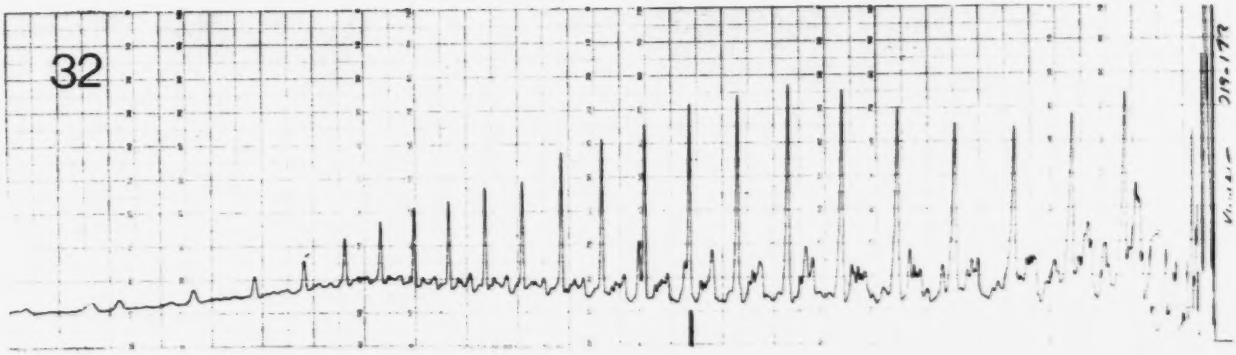
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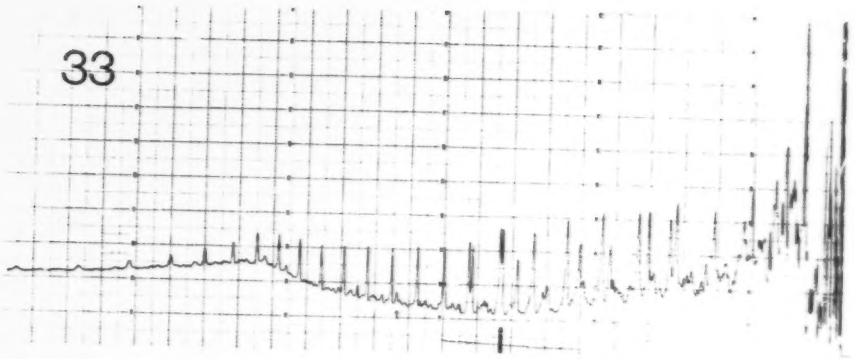
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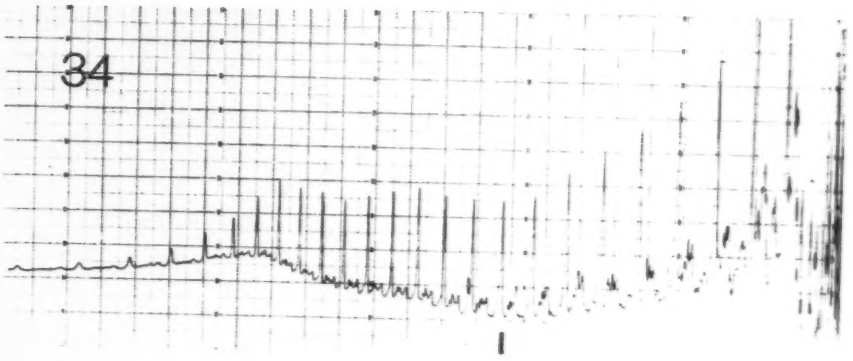
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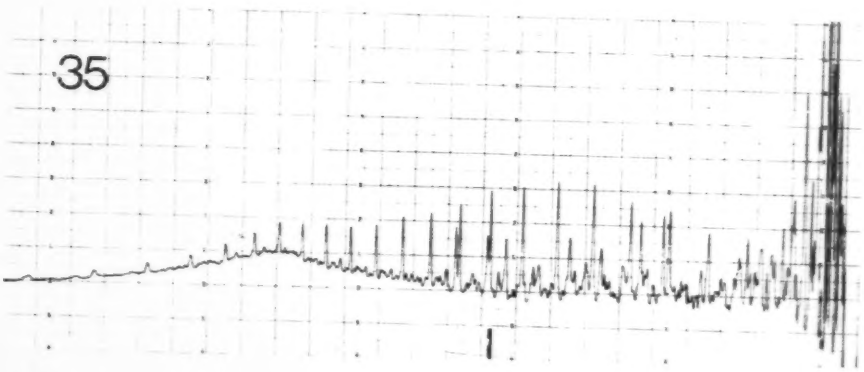
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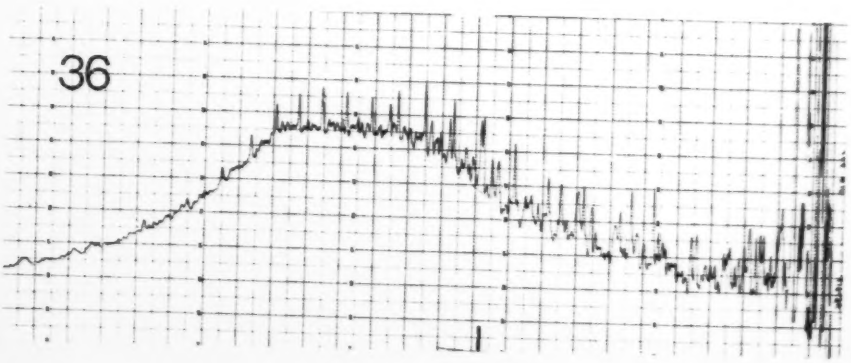
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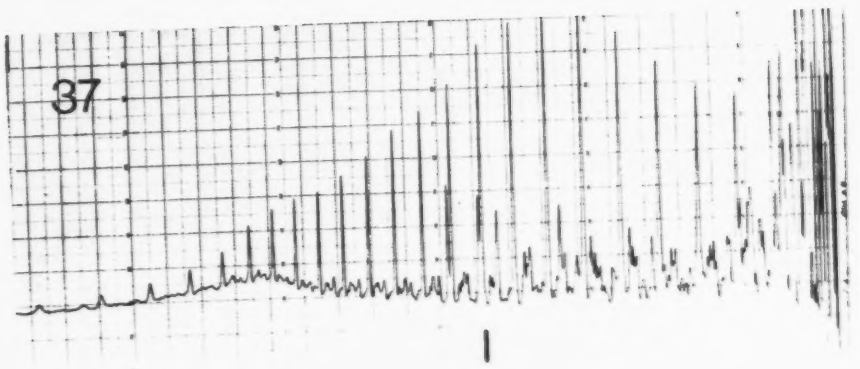
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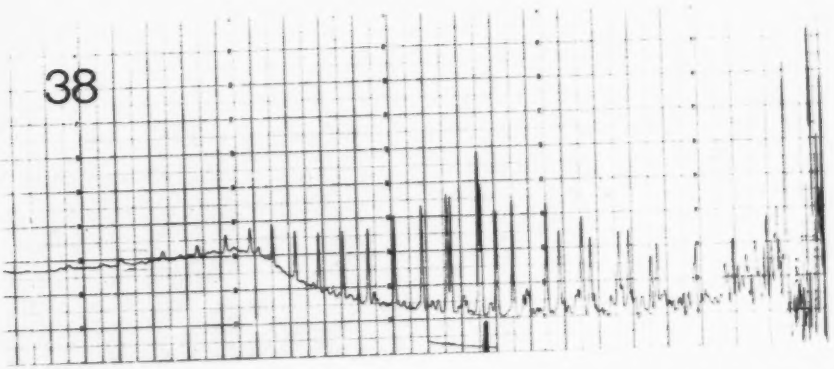
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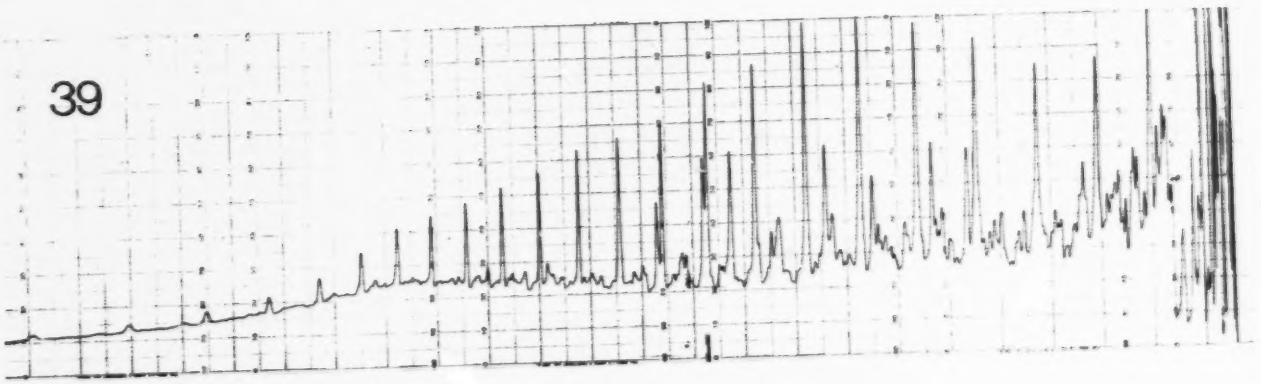
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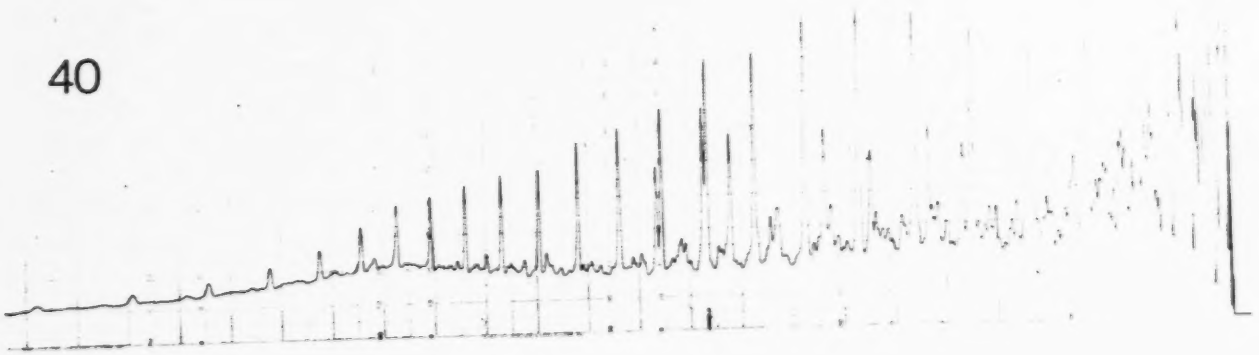
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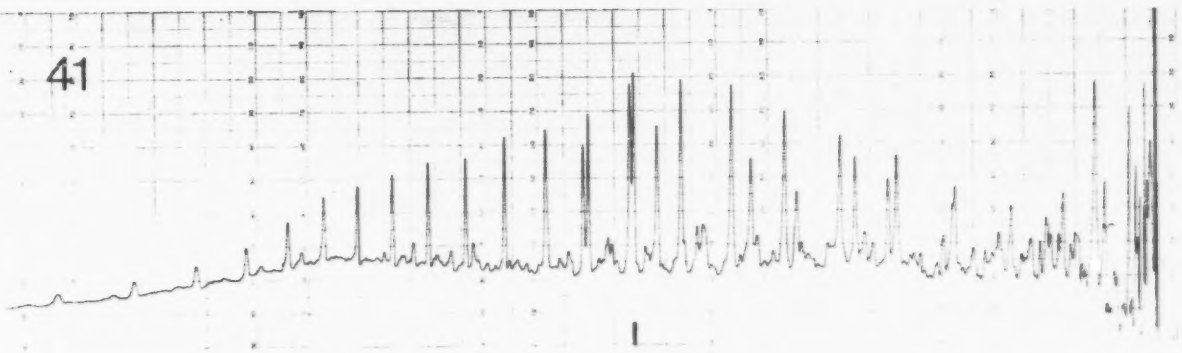
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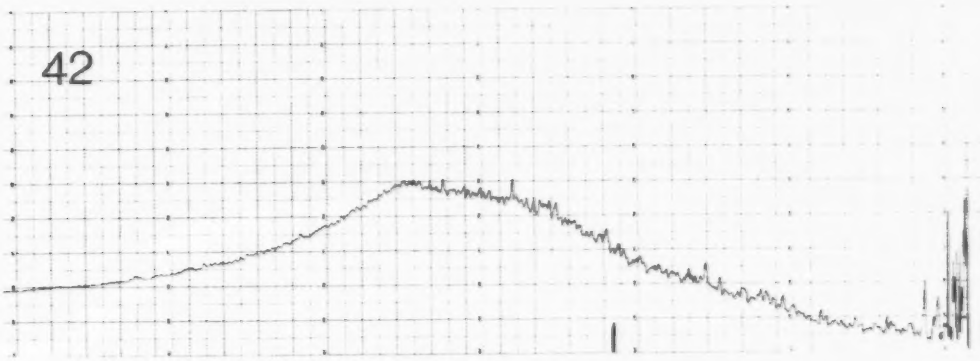
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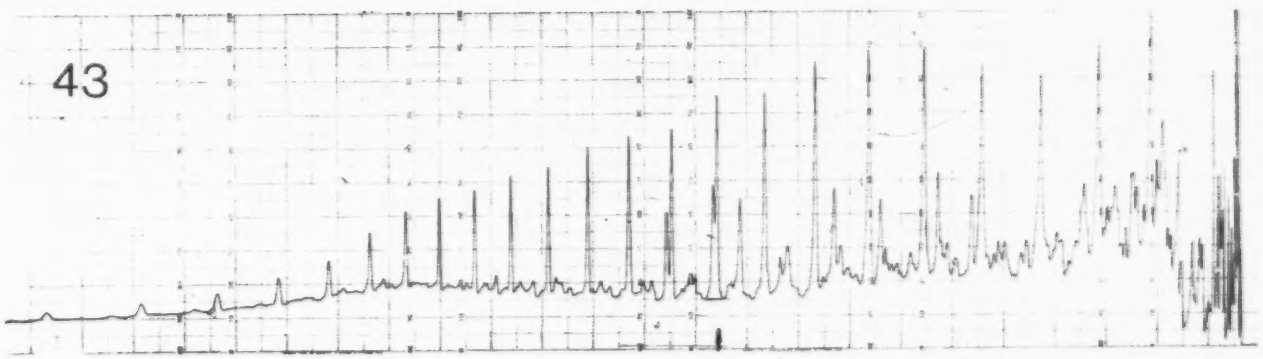
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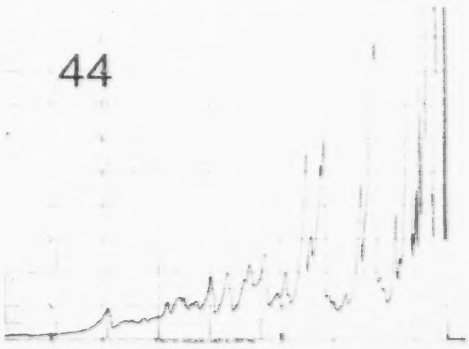
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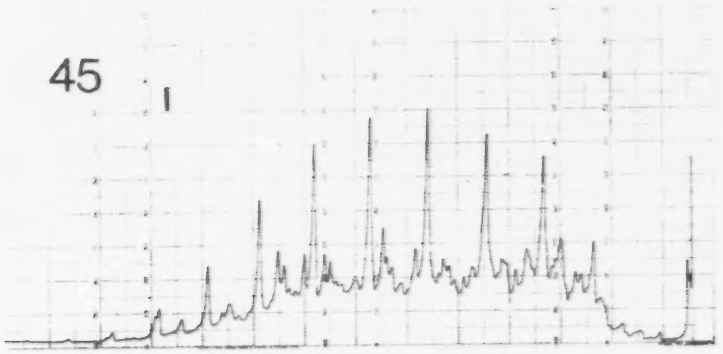
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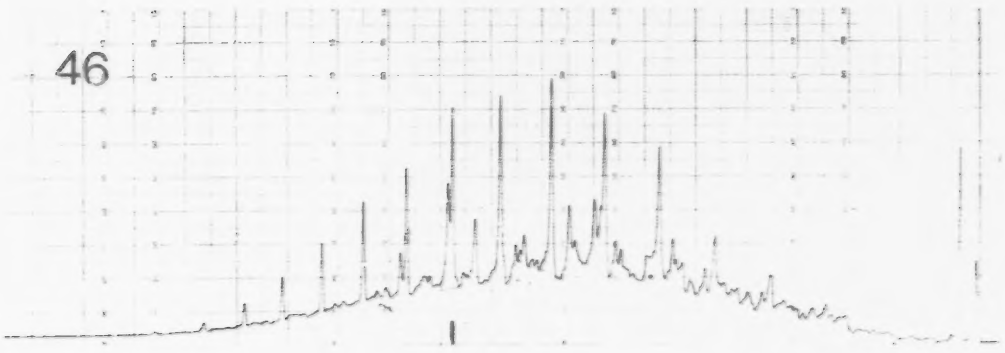
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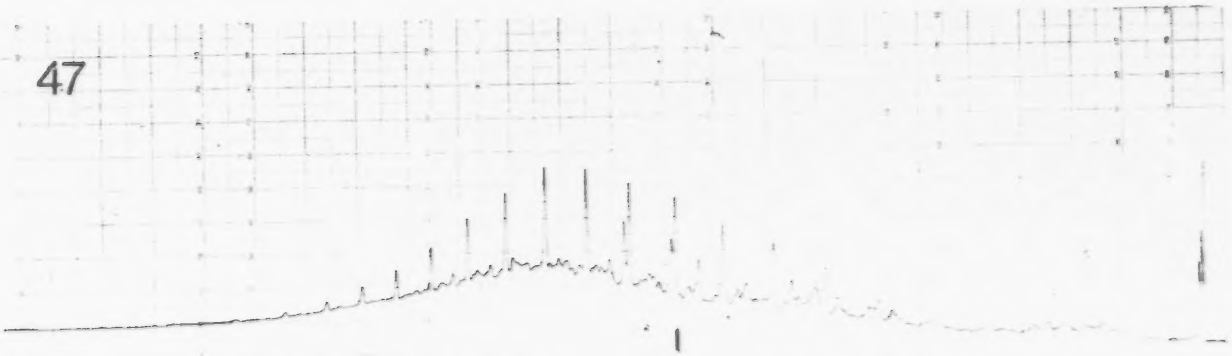
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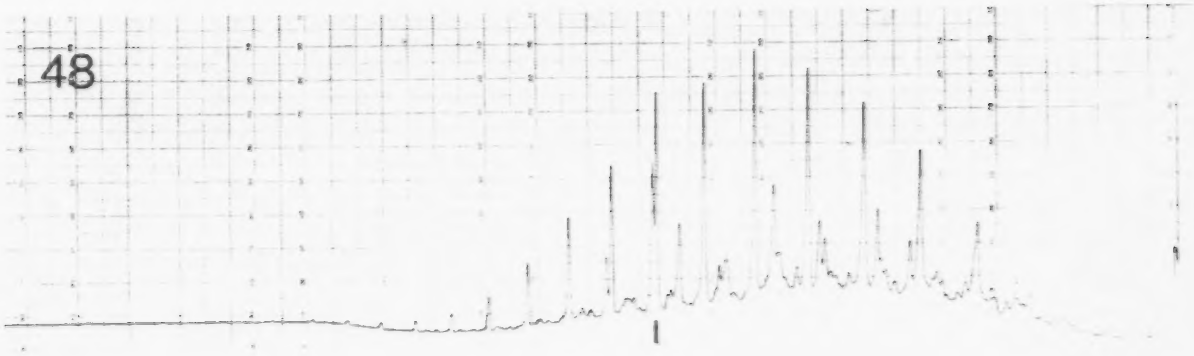
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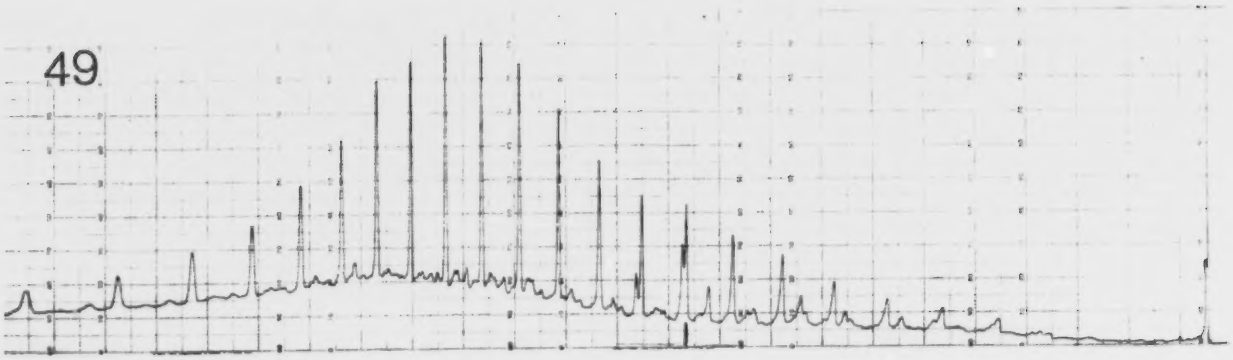
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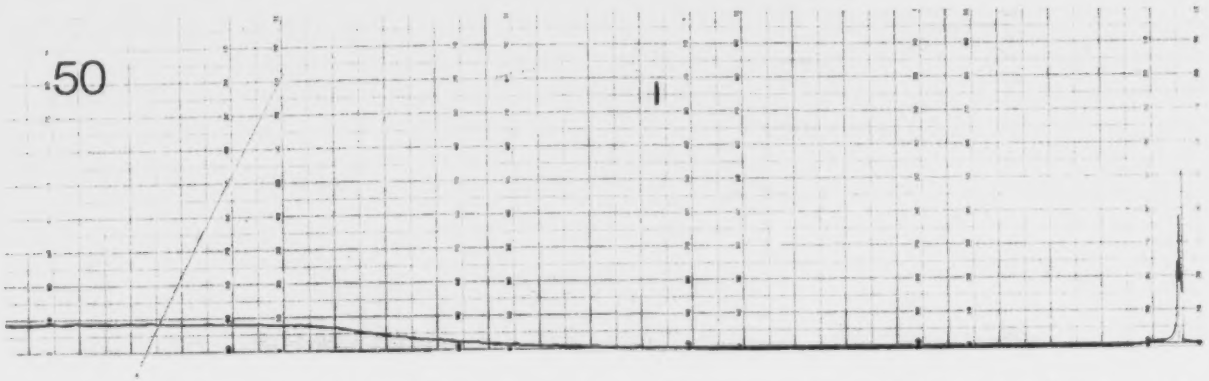
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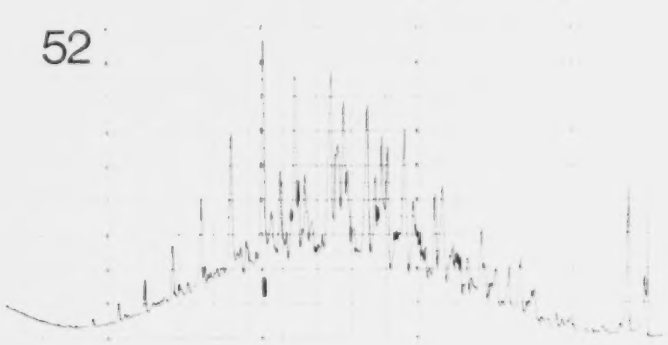
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APPENDIX 3

INTERCALIBRATION OF ANALYSES OF RECENTLY BIOSYNTHESIZED HYDROCARBONS AND PETROLEUM HYDROCARBONS IN MARINE LIPIDS

This Appendix describes briefly intercalibrating analyses of recently biosynthesized hydrocarbons and petroleum hydrocarbons in marine lipids. A detailed report of this intercalibration exercise will be available in the fall of 1972 from the Woods Hole Oceanographic Institution. Participants in the intercalibration exercise were Drs. John Teal and John Farrington (Woods Hole Oceanographic Institution) and Dr. James Quinn (Graduate School of Oceanography, University of Rhode Island).

The evaluation of the geographical extent and the severity of petroleum contamination of the marine environment requires the comparison of data obtained in different laboratories using the same or different analytical methods. We have examined the validity of comparing data collected in our laboratories. A cod liver extract was spiked with a distillate fraction (287^{o} - 450^{o} C) of South Louisiana Crude Oil. Each laboratory then analyzed the sample by its own commonly used methods. The concentrations of recently biosynthesized and petroleum hydrocarbons per unit weight of lipid were reported. Our intention was not to reproducibly and accurately identify the source of the petroleum contamination. Indeed we expect that most of the samples we analyzed from the continental shelf and deep sea, if contaminated, would have been contaminated by more than one source. Even if they were contaminated by only one source, the

presence of recently biosynthesized hydrocarbons, the probability of weathering of the petroleum hydrocarbons prior to incorporation into the sample and the probability of not having suspect oil samples would make it extremely difficult to identify the source.

The general method employed in each laboratory was to saponify the lipid extract, isolate the non-saponifiable lipids, and then isolate the hydrocarbons from the non-saponifiable lipids using thin layer or column chromatography. The hydrocarbons were then analyzed by gas chromatography on 7.5 ft. or 10 ft. 3% Apiezon L on Chromasorb W columns with the column temperature programmed from 80° or 100° C to 280° or 290° C at 5° or 6° per minute. Quantities of hydrocarbons were determined by comparing the detector signal of an internal standard n-alkane spike with the detector signal from the sample hydrocarbons, or by comparing the sample signal with the signal of a known quantity of an n-alkane analyzed on the same day using the same procedures.

The results are reported in the accompanying table and demonstrate that our interlaboratory precision is sufficient to allow valid comparison of analyses of hydrocarbons in the boiling range. The results also demonstrate that for the same boiling range, each laboratory gave a fairly good estimate of the level of petroleum hydrocarbon contamination. It should be emphasized that the precision and accuracy would vary somewhat depending on the composition of the petroleum hydrocarbon mixture with respect to the relative concentration of the non-polar n-alkanes and naphthenes, and the more polar mono-, di-, and polyaromatic hydrocarbons. Further intercalibration analyses using a variety of petroleum products such as paraffinic crude oils, naphthenic crude oils, fuel oils, bunker

oils, lubricating oils--all spiked in various matrices such as water, sediment, lipid-rich and lipid-poor organisms--are needed to demonstrate the validity of interlaboratory comparisons of data.

INTERCALIBRATION OF HYDROCARBON ANALYSES

IDOE - 5

(μg hydrocarbons/g cod liver lipid)

	QUINN	TEAL	FARRINGTON	AVERAGE (s.d. (r.s.d.))*
PEAKS + COMPLEX MIXTURE**	373.3	437.7	406.9	406 26.3 (6.5%)
PEAKS	85.5	87.7	64.4	79.2 10.5 (13.3%)
COMPLEX MIXTURE	287.8	350.0	342.5	326.8 27.7 (8.5%)
PRISTANE	263.5	276.0	271.8	270.4 5.2 (1.9%)
SQUALENE	251.7	271.0	292.1	271.6 16.5 (6.1%)
TOTAL	888.5	984.7	970.8	948.0 42.5 (4.5%)

* s.d.--standard deviation; r.s.d.--relative standard deviation

** This was the estimate of petroleum hydrocarbons. The sample was spiked with 371.8 μg petroleum hydrocarbons/g cod liver lipid.

APPENDIX 4

Procedure for Cleaning Varian Capillary Injector

CAUTION: It is crucial not to allow particles to be flushed into the line leading to the column!

1. Turn off column oven and injector heaters and allow to cool several hours. Shut off air and hydrogen.
2. Remove the column and the instrument side panel. Disconnect the carrier gas supply line and the split exit line at the closest accessible point to the injector; be sure the proper lines are located and that no valves or flow controllers are between the disconnected line ends and the injector. Remove septum nut, septum and glass liner.
3. Step 2 makes accessible four openings into the injector unit: column connection, carrier gas inlet, injection port and split exit. Plug the gas inlet and split exit lines and connect a tight-fitting piece of teflon or polypropylene tubing to the column fitting; the other end should fit a syringe tip. Be sure the tubing and plugs used contain no soluble plasticizers. Flush several 5 cc portions of particle-free methylene chloride through the column connector and catch in a flask; inspect each washing for color and for fine glass particles.
4. Disconnect the tubing and tightly plug the column opening with a blind stainless steel plug furnished with the column. Flush solvent through the carrier gas line and out the injection port.
5. Cap the port tightly, using a piece of teflon or polyethylene behind the septum, and flush several rinsings of CH_2Cl_2 through the carrier

gas line and out the split exit line. This line is particularly dirty and should only be flushed in this direction.

6. Dry injector with gas stream, reassemble connections in original fashion, check all reconnected fittings for leaks with soap solution, reinstall liner and septum, and reheat with carrier gas flowing and column exit unplugged. When fully heated, reconnect column and reset gas flow rates if necessary.

APPENDIX 5

SOURCE - SPILL CORRELATION DATA FOR EPA TEST SERIES

Source/ Spill[a]	Oil Type and/or Spill Conditions[a]	Signal Intensity Ratios[b]					Reference Chromatogram
		Pris/ Phyt.	$n\text{-}C_{17}$/ Pris.	$n\text{-}C_{18}$/ Phyt.	$n\text{-}C_{17}$/ $n\text{-}C_{18}$	$n\text{-}C_{17}$/ B.G.	
Source	#2 Fuel Oil	3.45	1.36	3.16	1.49	6.56	-
Spill	24 hours, F.W., WGS	3.10	1.48	3.01	1.52	5.80	-
Spill	1 month, F.W.	3.00	1.49	3.45	1.30	9.32	-
Spill	1 month. S.W.	2.45	1.34	2.64	1.24	5.00	-
Source	So. Louisiana Crude	1.61	1.34	1.68	1.28	4.97	7,19
Spill	1 month, S.W., WGS	1.51	1.36	1.69	1.22	4.69	-
Spill	24 hours, F.W.	1.55	1.34	1.71	1.21	4.78	8
Spill	1 month, F.W.	1.46	1.35	1.66	1.18	4.89	9
Spill	1 month, S.W.,S.H.[c]	1.52	1.25	1.59	1.19	3.60	-
Spill	24 hours, F.W.,COR	1.67	1.31	1.65	1.31	5.10	-
Spill	24 hours, F.W., MAG	1.62	1.40	1.77	1.29	5.00	-
Spill	1 month, S.W.	1.61	1.29	1.67	1.25	5.19	-
Spill	72 hours, F.W.	1.64	1.36	1.62	1.37	4.35	-
Spill	1 month, S.W., MAG	1.55	1.36	1.63	1.30	4.94	-
Spill	1 month, S.W., COR	1.58	1.31	1.66	1.25	5.15	-
Source	Bunker "C"	0.95	1.22	1.25	0.94	2.57	-
Spill	1 month, S.W., S.H.[c]	0.78	1.10	1.06	0.80	2.21	-
Spill	1 month, S.W.	0.77	1.06	1.04	0.78	2.38	-

Appendix 5 (Continued)		Pr/Ph	17/Pr	18/Ph	17/18	17/BG	
Source	Arabian Crude, Light	0.94	1.93	1.58	1.15	5.90	37
Source	#4 Fuel Oil	1.44	0.87	0.97	1.28	1.29	-
Source	Mix: #6 F.O., Arabian, So. La.	1.46	1.13	1.41	1.17	2.30	-
Source	Agha Jari Crude	0.92	1.90	1.56	1.13	5.39	22
Source	West Texas Sour Crude	1.12	1.55	1.42	1.22	5.55	-
Source	NORCO #2 Fuel Oil	1.14	1.16	1.34	0.99	2.53	-
Source	Lagonillas (LAGO) Crude	1.00	1.01	1.08	0.94	1.48	36
Spill	1 month, S.W., SH[c]	0.95	0.96	0.96	0.94	1.45	-
Spill	1 month, S.W.	0.99	0.91	0.99	0.91	1.37	-
Spill	1 month, S.W., WGS	0.98	0.91	0.98	0.91	1.38	-
Source	Navy Special Fuel Oil	1.05	1.31	1.26	1.09	3.55	-
Source	Chevron Ventura Crude	1.29	0.84	0.98	1.10	5.23	38
Source	NORCO #5 Fuel Oil	1.54	1.40	1.84	1.17	4.26	-
Source	Waste crankcase Oil	-	-	-	-	0/0	51
Source	#6 Fuel Oil	1.37	1.72	2.46	0.96	5.58	-
Spill	72 hours, F.W.	1.39	1.61	2.47	0.90	5.71	-
Spill	1 month, F. W.	1.31	1.68	2.39	0.92	5.62	-
Spill	24 hours, F.W., SH[c]	1.37	1.54	2.07	1.02	3.50	-
Spill	24 hours, F.W.	1.36	1.69	2.45	0.93	5.53	-
Source	La Rosa Crude	0.96	1.34	1.18	1.09	2.73	35

Appendix 5 (Continued)		Pr/Ph	17/Pr	18/Ph	17/18	17/BG	
Spill	1 month, S.W., COR	0.93	1.23	1.10	1.04	2.57	-
Spill	1 month, S.W., WGS	0.92	1.25	1.10	1.04	2.75	-
Spill	1 month, S.W.	0.89	1.29	1.12	1.02	2.81	-
Spill	1 month, S.W., SH[c]	0.92	1.19	1.10	1.00	2.03	-
Spill	1 month, S.W., MAG	0.95	1.42	1.29	1.05	3.42	-
Source	Bachaquero Crude	Not Supplied.....					-
Spill	72 hours, F.W.	0.85	0.95	0.98	0.83	1.00	-
Spill	1 month, F. W.	0.86	0.92	0.95	0.83	0.99	-
Spill	24 hours, F.W.	0.85	0.96	0.99	0.84	1.02	42

a Each EPA simulated spill is listed under its parent source. The duration of the "weathering" is indicated. "FW" and "SW" indicate fresh and salt water, respectively. The other abbreviations indicate a spill control chemical that was added:

SH--Shell Herder, WGS--W. G. Smith] Surface Collecting Agents
MAG--Magnus dispersant (oil base)
COR--Corexit dispersant (water base)

b Ratios given are generally averages of 2-4 determinations.

c The spill control chemical SH gave an interference which can be corrected by column chromatography. Spills treated with SH were not corrected (except for Lagonillas; 1 month, SW, SH) and the ratios given reflect this.